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PRACTICAL MANUAL

FIRST YEAR B.PHARM (II-SEMESTER)

SUBJECTS: HUMAN ANATOMY AND PHYSIOLOGY II

PHARMACEUTIAL ORGANIC CHEMISTRY-I

BIOCHEMISTRY

Name :

Course : Bachelor of Pharmacy

Semester: II semester

INDEX

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SUBJECT: HUMAN ANATOMY AND PHYSIOLOGY II 207 P

PREPARED BY

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EXPT No: 1

DATE:

INTEGUMENTARY SYSTEM AND SPECIAL SENSES

AIM

To study the integumentary system and special senses.

INTEGUMENTARY SYSTEM

Integumentary system consists of a group of organs working together to perform specific activities. The integumentary system is composed of the skin, hair, oil and sweat glands, nails, and sensory receptors. The integumentary system helps maintain a constant body temperature, protects the body and provides sensory information about the surrounding environment. The skin completely covers the body and is continuous with the membranes lining the body orifices. It protects the underlying structures from injury and from invasion by microbes. It contains sensory (somatic) nerve endings of pain, temperature and touch. It is involved in the regulation of body temperature. The superficial, thinner portion, which is composed of epithelial tissue, is the epidermis. The deeper, thicker connective tissue part is the dermis. The skin has a surface area of about 1.5 to 2 m2 in adults and it contains glands, hair and nails. Between the skin and underlying structures there is a layer of subcutaneous fat.

EPIDERMIS

The epidermis is the most superficial layer of the skin and is composed of stratified keratinized squamous epithelium which varies in thickness in different parts of the body.

It is thickest on the palms of the hands and soles of the feet. There are no blood vessels or nerve endings in the epidermis, but its deeper layers are bathed in interstitial fluid from the dermis, which provides oxygen and nutrients, and is drained away as lymph. Epidermis contains following strata or layers-

1. Stratum cornium
2. Stratum lucidum
3. Stratum granulosam
4. Stratum germinativum
5. Stratum basale

DERMIS

The dermis is tough and elastic. It is formed from connective tissue and the matrix contains collagen

fibres interlaced with elastic fibres. The structures in the dermis are:

1. blood vesse
2. lymph vessels
3. sensory (somatic) nerve endings
4. sweat glands and their ducts
5. hairs, arrector pili muscles and sebaceous gland

FUNCTIONS OF THE SKIN

Protection

Regulation of body temperature

Formation of vitamin D

Sensation

Absorption

Excretion

RESULT:

SPECIAL SENSES

THE EAR

The ear is the organ of hearing. Ear is supplied by 8th cranial nerve and is stimulated by vibrations caused by sound waves.

Ear has three parts

1. The internal ear
2. The middle ear or tympanic ear
3. The external ear

The External Ear

It is divided into two parts the auricle and external acoustic meatus.The auricle is the expanded portion. It projects from the side of the head. The lobule is the soft part at the lower extremity.The external acoustic meatus is a tortes canal about 2.5cm long. It extends from the auricle to the tympanic membrane. It is slightly‘s’ shaped it transmit the sound waves perpendicularly to the membrane. It prevents the mechanical injury to the tympanic membrane from outside.

The Middle Ear

It is an irregular shaped cavity. The middle ear shows the presence of three minute bones known as the auditory ossicles. These bones form a series of movable joints with tympanic membrane. The auditory ossicles are the malleus, incus and stapes. The malleus lays on lateral the aspect of the middle ear. It looks like a hammer and presents a head, neck and handle. The handle of malleus is in contact with the head forms a movable joint with incus. The incus is the middle bone it looks like anvil it posses a body and two processes. The body articulates with the malleus. Stapes is the most medial of the ossicles. The head joints with the incus.

The Internal Ear

The internal part of the ear contains organs of hearing grouped as bony labyrinth and membranous labyrinth.The bony labyrinth is larger than membranous labyrinth and space between is filled with a fluid known as perilymph. The bony labyrinth includes vestibules, cochlea, and semicircular canals. The vestibule is the external part of the cochlea it looks like a snail shell. It represents a broad base.The three semicircular canal are arranged in such a way that one is situated in each of three planes of space. The semi circular canals are continues with the vestibule.

The membraneous labyrinth is small and presents a vestibule, cochlea and three semicircular canals. The membraneous cochlea is called the duct of cochlea. The inferior aspect is formed by the membrane which presents the special cells surrounded by minute hair like processes in the hair cells. These cells and nerve fibres combine to form organ of corti.

The Physiology of Hearing

The vibration travels as a succession of waves known as sound waves. The waves travel at 335 m/s. Ear picks up these vibrations and direct them towards the cochlea. The organ of corti is adapted to transmit them as nerve impulse by the 8^{th} cranial nerve where they are perceived as sound.

THE EYE

The eye is a special organ of the sense of sight. It is supplied by the optic nerve. The eye is situated in the orbital cavity. It is spherical in shape and is about 2.5cm in diameter. The bony walls of the orbit and fat help to protect the eye from injury

The three layers of tissue constitute the walls of the eye

- *Sclera, cornea* – The outer fibrous layer
- *Ciliary body, choroid* – The middle vascular layer
- *Retina* – The inner nervous layer

The other structure include the lens, the aqueous fluid and the vitreous body

Sclera :- The sclera or white of the eye forms the outermost layer of tissue of the eye ball. It is continuous with transparent cornea anteriorly. The sclera maintains the form and shape of eye. The cornea is convex is involved in the refraction of light rays to focus them on retina.

Choroid:-it lies posterior to sclera. It is deep chocolate brown in colour. Light Enters the eye through pupil, stimulate the nerve ending in the retina and then the rays are absorbed by choroid.

Ciliary body:-It is a continuation of the choroid. It gives attachment to a fine ligament called as suspensory ligament. The contractions and relaxations of ciliary muscle change

the thickness of the lens. It is associated with the secretion of aqueous humour and vitreous humour.

The iris:-It lies behind the cornea and extends from the ciliary body. It is a circular body composed of pigment cells. The centre aperture of the iris is called pupil. The size of pupil varies according to intensity of light. The iris is the coloured part of the eye, and its colour depends upon the number of pigment

The lens:-It is a circular biconvex transparent body suspended by suspensory ligaments. It lies behind the pupil and is highly elastic.

The retina:-It is the innermost layer of the wall of the eye. It is composed of several layers which are highly sensitive to light known as layers of rods and cones. The retina has a purple tint due to the presence of rhodopsin in rods.

SENSE OF SMELL

The nasal cavity

It has a dual function: a passageway for respiration and sense of smell.

Olfactory nerves (first cranial nerves) These are the sensory nerves of smell.

They originate as specialised olfactory nerve endings (chemoreceptors) in the mucous membrane of the roof of the nasal cavity above the superior nasal conchae.

On each side of the nasal septum nerve fibres pass through the cribriform plate of the ethmoid bone to the olfactory bulb where interconnections and synapses occur.

From the bulb, bundles of nerve fibres form the olfactory tract which passes backwards to the olfactory area in the temporal lobe of the cerebral cortex in each hemisphere where the impulses are interpreted and odour perceived.

Physiology of smell

All odorous materials give off volatile molecules, which are carried into the nose with the inhaled air and stimulate the olfactory chemoreceptors when dissolved in mucus.

The air entering the nose is warmed and convection currents carry eddies of inspired air to the roof of the nasal cavity.

'Sniffing' concentrates volatile molecules in the roof of the nose. This increases the number of olfactory receptors stimulated and thus the perception of the smell.

The sense of smell may affect the appetite. If the odours are pleasant the appetite may improve and vice versa. When accompanied by the sight of food, an appetising smell increases salivation and stimulates the digestive system.

SENSE OF TASTE

TONGUE

Taste buds contain sensory receptors (chemoreceptors) that are found in the papillae of the tongue and widely distributed in the epithelia of the tongue, soft palate, pharynx and epiglottis.

They consist of small sensory nerve endings of the glossopharyngeal, facial and vagus nerves (cranial nerves VII, IX and X).

Some of the cells have hair-like microvilli on their free border, projecting towards tiny pores in the epithelium.The sensory receptors are stimulated by chemicals that enter the pores dissolved in saliva. Nerve impulses are generated and conducted along the glossopharyngeal, facial and vagus nerves before synapsing in the medulla and thalamus.

Their final destination is the taste area in the parietal lobe of the cerebral cortex where taste is perceived.

PHYSIOLOGY OF TASTE

Four fundamental sensations of taste have been described — sweet, sour, bitter and salt.

This is probably an oversimplification because perception varies widely and many 'tastes' cannot be easily classified. However, some tastes consistently stimulate taste buds in specific parts of the tongue • sweet and salty, mainly at the tip • sour, at the sides • bitter, at the back.

The sense of taste triggers salivation and the secretion of gastric juice. It also has a protective function, e.g. when foul-tasting food is eaten then reflex gagging or vomiting may be induced.

EXPT NO: 2

DATE

NERVOUS SYSTEM

Nervous system can be described as follows

A) The central nervous system consisting of the brain and spinal cord

B) The peripheral nervous system consisting of 1) 31 Pair of spinal nerves 2) 12 Pair of cranial nerves 3) Autonomic nervous system

1) Brain

Structure: It consist about 1/5th of the body weight.It lies within cranial cavity

The structure forming the brain are a) The cerebrum or fore brain b) The mid brain c) The pons of varoli-The brain stem d) The medulla oblongata e) The cerebellum

A) Cerebrum

Structure It constitute the largest part of brain.It occupies the anterior and middle cranial fossae. Each hemisphere of the cerebrum is divided for descriptive purposed into lobes which have the names of the bones of the cranium under which they are a) Frontal b) Parietal c) Temporal d) Occipital.

It shows two types of areas- motor and sensory

Funtions

- The mental activities involved in memory. intelligence sense of responsibility, Thinking, reasoning, moral sense and learning attributes to the higher centers
- Sensory perception which include the perception of pain, temperature, touch and special senses of sight, hearing, taste and smell
- Initiation and control of the contraction of voluntary muscle

B) Mid Brain

Structure: - It is the area of brain between the cerebrum above and the poX`n's varoli below

- It consist of group nerve cell and nerve.fibres which connect the cerebrum with lower part of the cerebrum and spinal cord

Function

It maintains the balance of the body.

C) The Pons Varoli

Structure: - It is situated in front of the cerebellum below the mid brain and above the medulla oblongata.Itconsist of nerve fibers

D) The Medulla Oblongata

Structure:-It extend from the pons varoli above and it continuous with spinal cord below

- It is about 2.5 cm long and is shaped like pyramid with it is base upward
- It is within the cranium

Functions

- The vital centre's associated with autonomic reflex activity are present in its deeper structure. these are

a) Cardiac centre -which regulate and control rhythmically, automatically, conductivity, contractility of myocardium

b) The respiratory centre- regulate rate and depth of respiration

c) Vaso motor centre control size of blood vessels

d) The reflex centre vomiting, sneezing, coughing and swallowing

E) Cerebellum

Structure:-

- It behind the pons of varoli
- It is ovoid in shape
- It present two hemisphere which are separated by narrow mediator-strip known as vermis
- **Function**

It is concerned with voluntary muscle movement and balance

Spinal Cord

It is defined as nervous tissue link between the brain and peripheral organs of the body.

Structure:-

- It is elongated, almost cylindrical part of the CNS
- It lies within the vertebral canal of vertebral column.

- It is surrounded by dura and arachnoids matters
- It is continuous with medulla oblongata above and extends from the upper border of after to the lower border of the first lumbar vertebrae

EXPT No: 3

DATE:

THE ENDOCRINE SYSTEM

Endocrine glands consist of groups of secretory cells surrounded by an extensive network of capillaries which facilitates diffusion of hormones (chemical messengers) from the secretory cells into the bloodstream. They are commonly referred to as the ductless glands because the hormones are secreted and diffuse directly into the bloodstream.

A hormone is formed in one organ or gland and carried in the blood to another organ (target organ or tissue), Most hormones are synthesised from amino acids (amines, polypeptides and protein or are cholesterol-based lipids (steroids). Homeostasis of the internal environment is maintained partly by the autonomic nervous system and partly by the endocrine system. The autonomic nervous system is concerned with rapid changes, while hormones of the endocrine system are mainly involved in slower and more precise adjustments.

The endocrine system consists of a number of distinct glands and some tissues in other organs. Although the hypothalamus is classified as a part of the brain and not as an endocrine gland it controls the pituitary gland and has an indirect effect on many others.

The endocrine glands are:

• 1 pituitary gland • 2 adrenal (suprarenal) glands) • 1 thymus gland • 1 pineal gland or body • 1 thyroid gland • 4 parathyroid glands • the pancreatic islets (islets of Langerhans) • 2 testes in the male • 2 ovaries in the female

Glands/hormone	Cell/tissue source	Major functions
(a) Primary endocrine organs		
Hypothalamus		
Corticotropin-releasing hormone (CRH)	Paraventricular nuclei (PVN)	Stimulates release of ACTH and B-endorphin from anterior pituitary
Gonadotropin-releasing hormone (GNRH)	Preoptic area; anterior hypothalamus	Stimulates release of FSH and LH from anterior pituitary
Luteinizing hormone-releasing hormone (LHRH)	Nuclei; medial basal hypothalamus (rodents and primates); arcuate nuclei (primates)	Stimulates release of LH from anterior pituitary
Somatostatin (growth hormone-inhibiting hormone)	Anterior periventricular nuclei	Inhibits release of GH and TSH from anterior pituitary inhibits release of insulin and glucagon from pancreas
Melanotropin-release inhibitory factor (Dopamine)	Arcuate nuclei	Inhibits the release of MSH (no evidence of this peptide in humans)
Neuropeptide Y (NPY)	Arcuate nuclei	Regulation of energy balance
Neurotensin	Arcuate nuclei	Regulation of energy balance
Orexin A and B	Lateral hypothalamic area	Regulation of energy balance/ food intake
Thyrotropin-releasing hormone	Paraventricular nuclei (PVN)	Stimulates release of TSH and PRL from anterior pituitary

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Glands/hormone	Cell/tissue source	Major functions
Pituitary		
Adrenocorticotropic hormone (ACTH)	Anterior pituitary	Stimulates synthesis and release of glucocorticoids
Follicle-stimulating hormone (FSH)	Anterior pituitary	Stimulates development of ovarian follicles and secretion of estrogens; stimulates spermatogenesis
Growth hormone (GH)	Anterior pituitary	Mediates somatic cell growth
Luteinizing hormone (LH)	Anterior pituitary	Stimulates Leydig cell development and testosterone production in males; stimulates corpora lutea development and production of progesterone in females
Melanocyte-stimulating hormone (MSH)	Anterior pituitary	Affects memory; affects skin color in amphibians
Prolactin (PRL)	Anterior pituitary	Many actions relating to reproduction, water balance, etc.
Thyroid-stimulating hormone or thyrotropin (TSH)	Anterior pituitary	Stimulates thyroid hormone secretion
Oxytocin	Posterior pituitary	Stimulates milk letdown and uterine contractions during birth
Vasopressin or antidiuretic hormone ADH or AVP	Posterior pituitary	Increases water reabsorption in kidney

Adrenal gland		
Mineralocorticoids		
Aldosterone	Zona glomerulosa of adrenal cortex	Sodium retention in kidney
11-Deoxycorticosterone (DOC)	Zona glomerulosa of adrenal cortex	Sodium retention in kidney
Glucocorticoids		
Cortisol (hydrocortisone)/ corticosterone	Zona fasciculata and z. reticularis of adrenal cortex	Increases carbohydrate metabolism; antistress hormone
		Increased carbohydrate metabolism; antistress hormone
Dehydroepiandro-sterone DHEA	Zona reticularis of adrenal cortex	Weak androgen; primary secretory product of fetal adrenal cortex
Epinephrine or adrenaline (EP)	Adrenal medulla	Glycogenolysis in liver; increases blood pressure
Norepinephrine or noradrenaline (NE)	Adrenal medulla	Increases blood pressure
Thymus (Thymocytes)	Thymosin, thymulin and thymopoietin	Proliferation/differentiation of lymphocytes

Pineal gland

Melatonin	Pinealocytes	Affects reproductive and circadian control of bodily functions
Thyroid/parathyroid		
Thyroxine or tetraiodothyronine (T4) Triiodothyronine (T3)	Follicular cells	Regulate oxidation and basic metabolic rates in tissue; learning associated neuroplasticity
Calcitonin (CT)	C-cells of thyroid	Lowers serum Ca^{2+} levels
Parathyroid hormone (PTH)	Parathyroid gland	Stimulates bone resorption; increases serum Ca^{2+} levels

Pancreas (Islet cells of Langerhans)

Glucagon	a-cells	Glycogenolysis in liver
Insulin	b-cells	Glucose uptake from blood; glycogen storage in liver
Somatostatin	d-cells	Inhibits insulin and glucagon secretion
Pancreatic polypeptide (PP)	Peripheral cells of pancreatic islets	Effects on gut in pharmacological doses

Testes		
Androstenedione	Leydig cells	Male sex characters
Dihydrotestosterone (DHT)	Seminiferous tubules and prostate	Male secondary sex characters
Testosterone	Leydig cells	Spermatogenesis; male secondary sex characters
Ovary		
Estrogen	Follicles (also in brain regions like hippocampus, hypothalamus, prefrontal cortex, amygdala)	Uterine and other female tissue development Enhances cognition, neuroprotective
Progesterone	Corpora lutea, placenta	Uterine development; mammary gland development; maintenance of pregnancy

RESULTS:

EXPT No: 4

DATE:

DEMONSTRATION OF NEUROLOGICAL EXAMINATION

AIM:

To find the ability to tell whether objects are cold, hot, smooth or rough by given subject.

Principle

To demonstrate the different types of receptors in the skin, gather a number of objects that are:

- smooth (an apple)
- rough (sand paper; rock)
- cold (ice)
- warm (a sun-warmed piece of metal)

Tell someone to close their eyes (or use a blindfold) and touch the items to the person hands or fingers. Ask to concern what you feel? Based on the questions and answers will be help to find the neurological examination, which correlated to receptor activation and capacity of impulse generation towards to CNS.

Procedure

1. Healthy volunteer‘s eye was closed by using cloth.
2. Kept some products
3. Inform them to sense the products by touch
4. Identify the products details
5. Note the time of identification and identified products are correct or not correct

Report:

EXPT No: 5

DATE:

DEMONSTRATION OF THE FUNCTION OF OLFACTORY NERVE

AIM:

To demonstrate the function of olfactory nerve

REQUIREMENT:

Orange oil, fish liver oil, clove oil and rose water.

PRINCIPLE:

The olfactory nerve (First cranial nerve) carries the sensation of smell. The nerve arises from the olfactory mucosa and the sensation is carried from the nasal mucosa to the olfactory cortex of the brain.

PROCEDURE:

1. Asked the subject to sit comfortably on a stool.
2. Made sure that both the nostrils were patent and there was no inflammation of the nasal mucosa (no nasal obstruction due to common cold).
3. The subject was asked to close his eyes and one of his nostrils.
4. Removed the cap of the bottle containing clove oil and took the bottle close to the open nostril.
5. Asked the subject if he correctly perceived the smell.
6. Repeated the procedure with all the oils.
7. Asked the subject to close this nostril and open the other nostril and the procedure was repeated.
8. Compared the results of both the nostrils.
9. Asked the subject if he had any hallucination of smell.
10. Noted the result. The result was expressed as smell normal, reduced, absent or perverted, separately for each nostril.

RESULTS:

EXPT No: 6

DATE:

EXAMINATION OF THE DIFFERENT TYPES OF TASTE

AIM:

To examine the different types of taste

REQUIREMENT:

1.)Four small vials containing strong solutions that are sweet (sugar), salty (common salt), bitter (solution of quinine or chloroquine) and sour (lime juice or weak solutions of citric acid) separately.

2.)Four glass rods.

PRINCIPLE:

Taste, gustatory perception, or gestation is one of the five traditional senses that belongs to the gustatory system. Taste is the sensation produced when a substance in the mouth reacts chemically with taste receptor cells located on taste buds in the oral cavity, mostly on the tongue. Taste, along with smell (olfaction) and trigeminal nerve stimulation (registering texture, pain, and temperature), determines flavors of food or other substances. Humans have taste receptors on taste buds (gustatory calyculi) and other areas including the upper surface of the tongue and the epiglottis. The gustatory cortex is responsible for the perception of taste.

The tongue is covered with thousands of small bumps called papillae, which are visible to the naked eye. Within each papilla are hundreds of taste buds. The exception to this is the filiform papillae that do not contain taste buds. There are between 2000 and 5000 taste buds that are located on the back and front of the tongue. Others are located on the roof, sides and back of the mouth, and in the throat. Each taste bud contains 50 to 100 taste receptor cells.

The sensation of taste includes five established basic tastes: sweetness, sourness, saltiness, bitterness, and umami. Scientific experiments have proven that these five tastes exist and are distinct from one another. Taste buds are able

to differentiate among different tastes through detecting interaction with different molecules or ions.

PROCEDURE:

1. Asked the subject to protrude the tongue.
2. Dipped one glass rod in the sweet solution and different parts of the tongue of the subject was touched with the tip of the rod.
3. Asked the subject about the nature of taste he had sensed.
4. Similarly the procedure was repeated with salty, sour and bitter solutions by using separate glass rods for each solution.

RESULTS:

DISCUSSION:

The taste sensation testing should be carried out after considering the relative distribution of taste buds in the tongue for different modalities of taste. The taste buds for sweet are more concentrated at the tip, bitter at the back and salt and sour on the sides of the tongue.

EXPT No: 6

DATE:

DEMONSTRATION OF VISUAL ACUITY

AIM:

To determine the visual acuity

REFERENCE:

1. Text book of practical Physiology- G.K. Pal, Pravati Pal, IIIrd Edition, page no: 318.

REQUIREMENT:

1. Snellen's chart: Snellen's letters are depicted on a cardboard with eight rows of black letters of different forms. The topmost line can be read by a normal subject at a distance of 60 meters and subsequent lines at 36, 24, 18, 12, 9, 6 and 5 meters respectively.

PRINCIPLE:

A series of varying sizes are constructed in such a way that the top letter is visible to normal eyes at 60 meters and the subsequent lines at 36, 24, 18, 12, 9, 6 and 5 meters respectively. Visual acuity is recorded according to the formula V = d/D, where V is the visual acuity, d is the distance at which the letters are read and D is the distance at which the letters should be read.

PROCEDURE:

1. The subject was given proper instructions.
2. The subject was asked to sit at a distance of 6 meters from the chart (d=6) and close one of his eyes and read the chart with the other eye.
3. Noted the line up to which the subject was able to read comfortably.
4. He was asked to repeat the procedure with the opposite eye.

RESULTS:

EXPT No: 8

DATE:

DEMONSTRATION OF REFLEX ACTIVITY

Aim

To demonstrate the reflex activity by given subject

Principle

Nerve impulses follow routes through the nervous system called nerve pathways. Some of thesimplest nerve pathways consist of little more than two neurons that communicate across a single synapse. A reflex is a relatively simple motor response that does not involve a large number of interneurons (or association neurons). The simplest version is a mono-synaptic reflex that uses one sensory and one motor neuron (for example, the patellar or knee-jerk reflex). Most reflexes are polysynaptical (involving more than two neurons) and involve the activity of interneurons in the integration center. In these more complicated reflexes, impulses may travel up, down, and transversely in the spinal cord. Since there is synaptical delay in neural transmission at the synapses, the more synapses there are in the reflex pathway, the more time that is required to illicit the reflex. Reflexes are mediated over simplenerve pathways called reflex arcs. Reflex arcs have five essential components:

1. The receptor at the end of a sensory neuron reacts to a stimulus.

2. The sensory neuron conducts nerve impulses along an afferent pathway towards the CNS.

3. The integration center consistsof one or more synapses in the CNS.

4. A motor neuron conducts a nerve impulse along an efferent pathway from the integration center to an effector.

5. An effector responds to the efferent impulses by contracting (if the effector is a muscle fiber) or secreting a product (if the effector is a gland).

Reflexes can be categorized as either autonomic or somatic. Autonomic reflexes are not subject to conscious control, are mediated by the autonomic division of the nervous system, and usually involve the activation of smooth muscle, cardiac muscle, and glands.

Involuntary reflexes are very fast, traveling in milliseconds. The fastest impulses can reach 320 miles per hour. Somatic reflexes involve stimulation of skeletal muscles by the somatic or voluntary division of the nervous system. Reflex testing is an important diagnostic tool for assessing the condition of the nervous system. Distorted, exaggerated, or reflexes that are absent may indicate degeneration or pathology of portions of the nervous system, often before other signs are apparent. If the spinal cord is damaged, then reflex tests can help determine the area of injury. For example, motor nerves above an injured area may be unaffected, whereas motor nerves at or below the damaged area may be unable to perform the usual reflex activities.

Procedure

1.)To Select Healthy Volunteer

2.)Use sterile needle to prick the finger region and immediately observe the response.

3.) Use torch light to pass the light source towards eye and immediately observe the response.

4.) Note the reaction

Report:

EXPT No: 9

DATE:

DETERMINATION OF BODY TEMPERATURE

AIM:

To find out the body temperature using clinical thermometer

REFERENCE:

1. Goyal R.K , Patel N. M ,Practical anatomy and Physiology,13th edition page number 42-43

REQUIREMENTS:

Clinical thermometer, surgical spirit, cotton

PRINCIPLE:

Thermometry is an art of taking and recording the body temperature. Body temperature is recorded by using a clinical thermometer. It is a special type of mercury thermometer. It is different from ordinary thermometer in having a bend and a constriction on the capillary canal just above the mercury bulb. It also records the temperature within a limited range. It may have a Fahrenheit scale or a centigrade scale or both. The Fahrenheit scale normally ranges from 94^0 F to 108^0 F and the centrifuge scale is 35^0C to 40^0C. Humans cannot survive with a body temperature either below or above this scale. When the mercury bulb comes in contact with the body temperature, the mercury within it expands and raises in the capillary column depending up on the temperature. A direct reading of the body temperature can be obtained from the scale on the body of the thermometer. When mercury bulb is removed from its contact with the body level the falling down of mercury due to the contraction is prevented by the bend and constriction above the bulb. This enables to take correct reading. After the reading is taken the clinical thermometer is vigorously shaken as that it re allows mercury into the bulb. Before and after the use the clinical thermometer is washed with antiseptic lotion or with ordinary water

Body temperature can be measured in many locations on the body. The mouth, ear, armpit, and rectum are the most commonly used places.

Normal body temperature-97^0 F to 99^0 F

Average 98.6^0 F or 36^0 C to 37.2^0 C average37^0 C

Rectal temperature slightly higher than oral temperature (1^0 F> oral temperature)

Oral temperature- 36.11^0 C to 37.22^0 C

PROCEDURE:

The mercury bulb of the thermometer was placed in the mouth below tongue. The lips were closed and the breath was taken out a nd the readings were directly noted by reading the scale in terms of ^{0} F and ^{0} C.

REPORT

DISCUSSION:

Hypothermia –decrease in body temperature (30^0 C-25^0 C)

Hyperthermia – fever or pyrexia, increase in body temperature

FACTORS AFFECTING BODY TEMPERATURE

- Age – irregular in infants lower in old age
- Sex- body temperature is low in females
- Food, exercise and emotions- increase body temperature
- Toxins, injections – increase in body temperature
- Diurnal –highest in the evening and lowest in the morning

EXPT No: 10

DATE:

DEMONSTRATION OF POSITIVE AND NEGATIVE FEEDBACK MECHANISM

Positive feed-back mechanism

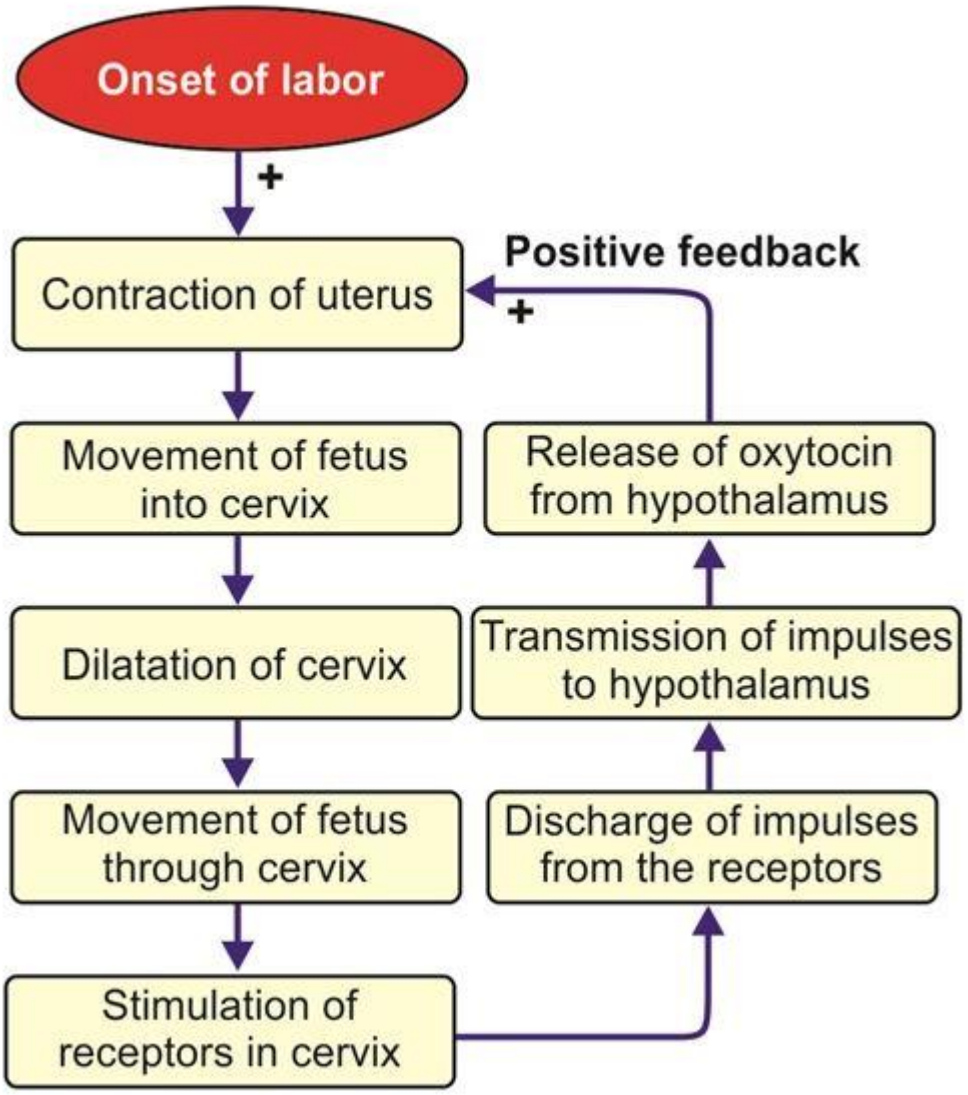
Positive feedback mechanism causes destabilizing effects in the body, so does not results in homeostasis. It is mainly responsible for amplification of the changes caused by the stimulus.

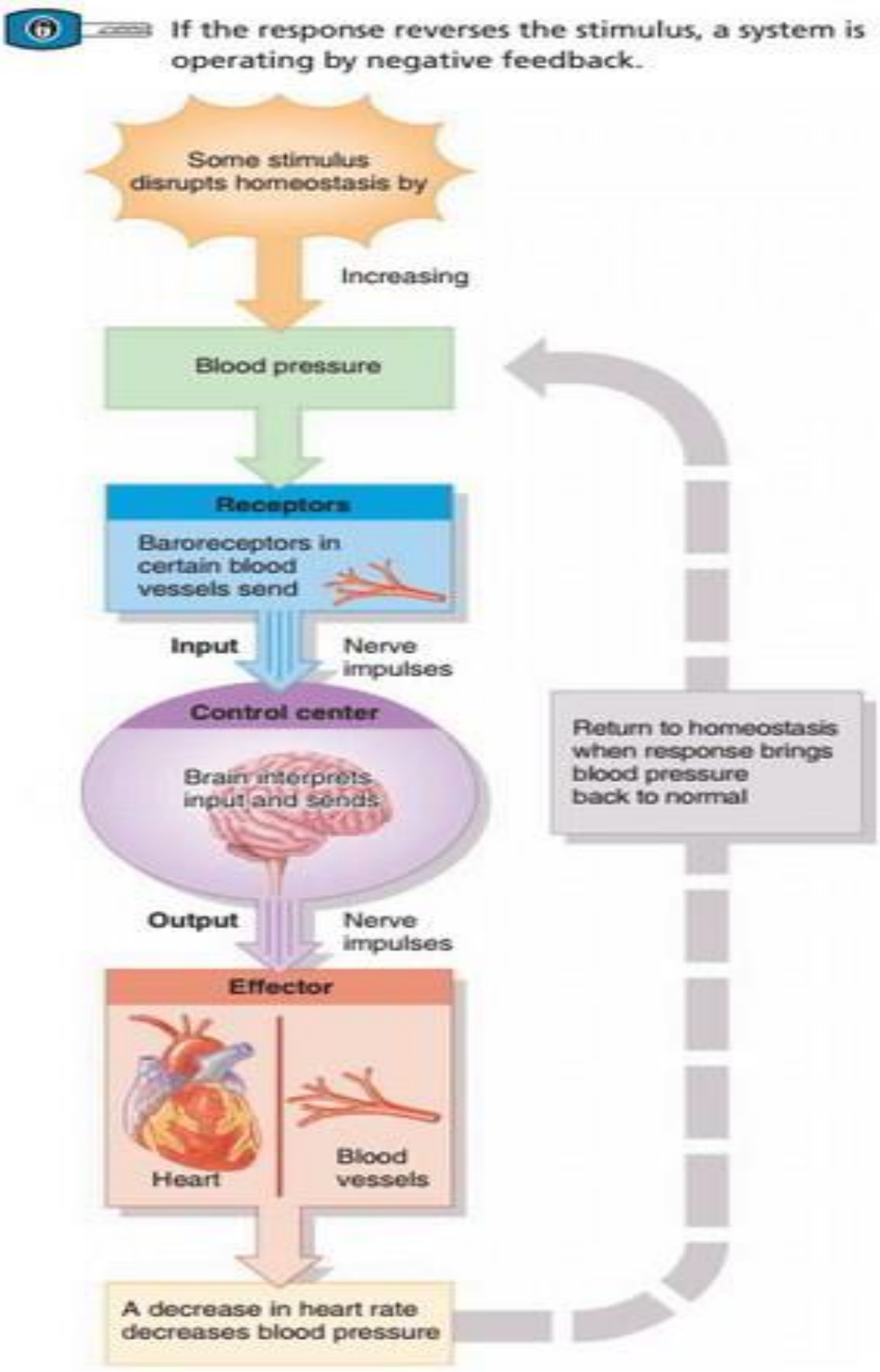
Positive feedback is relatively less common than negative feedback, since it leads to unstable condition and extreme state. Most positive feedback mechanisms are harmful and in somecases resulting in death. For example, if a person breathes air that has very high carbon dioxide content. The amount of oxygen in blood decreases while the concentration of carbon- dioxide in blood increases. This is sensed by carbon dioxide receptors, which cause the breathing rate to increase. So the person breathes faster, taking in more carbon dioxide, whichstimulates the receptors even more, so they breathe faster and faster which ultimately results in death.

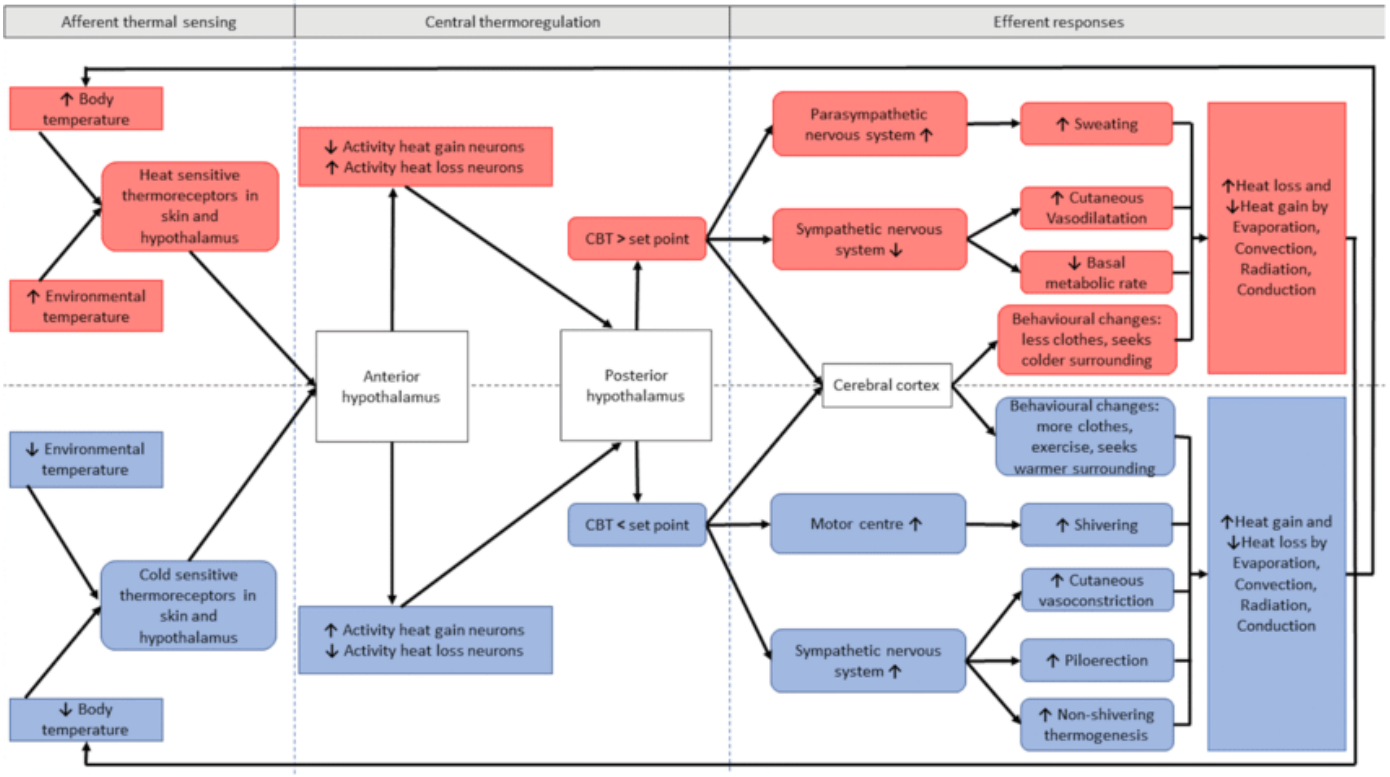
In some cases, the positive feed-back is very useful. Such as during blood clotting, fever, child birth, breast feeding etc. Positive feedback also plays a role in the contractions of the uterus during child birth. The contraction of uterine wall is caused by oxytocin hormone. In this case, stretching of the uterus by the fetus stimulates oxytocin release which results in contraction of uterus, and contraction causes further stretching and release of oxytocin; the cycle continues until the fetus is expelled from the uterus.

Negative feed-back mechanism

The receptors (sensory cells) are present on the body of vertebrates constantly monitors the reference point of internal environment. Any changes in the internal environment can activates the receptor cells, which relay messages to the control center (Brain or spinal cord). The control center determines the deviation and activates the effectors. Effectors are generally muscles or glands. The effectors are respond to the stimulus and corrects the reference point either by increasing or decreasing the activities. As soon as the system is corrected, the control center and effectors are turned off by the mechanism called Negative feed-back.


Onset of labor
+
Contraction of uterus
Positive feedback
+
Movement of fetus into cervix
Release of oxytocin from hypothalamus
Dilatation of cervix
Transmission of impulses to hypothalamus
Movement of fetus through cervix
Discharge of impulses from the receptors
Stimulation of receptors in cervix


If the response reverses the stimulus, a system is operating by negative feedback.
Some stimulus disrupts homeostasis by
Increasing
Blood pressure
Receptors
Baroreceptors in certain blood vessels send
Input
Nerve impulses
Control center
Brain interprets input and sends
Output
Nerve impulses
Effector
Heart
Blood vessels
A decrease in heart rate decreases blood pressure
Return to homeostasis when response brings blood pressure back to normal



In negative feed-back mechanism, changes occurring in the systems are automatically activates the corrective mechanism, which reverse the changes and bring back the system to the normal. The principle of thermostat is analog to the Negative feed-back mechanism. In thermostat, when the temperature exceeds the normal ranges, the receptor detects the changes and signals the control center of thermostat to turn off the heating plate, allowing the thermostat to cool down. When the thermostat cool down below the set point, it turn ON the heating plates, so the temperature starts rise again.

Report:

EXPT No: 11

DATE :

DETERMINATION OF TIDAL VOLUME AND VITAL CAPACITY

Aim

To determine lung tidal volume and vital capacity of given subject by using spirometer.

Principle

The amount of air that is flushed in and out of the lungs varies substantially depending on the conditions of inspiration and expiration. Several different respiratory volumes can be described. Specific combinations (sums) of these respiratory volumes are known as respiratory capacities. The instrument that is used to measure respiratory volumes is called a spirometer.During quiet breathing, the tidal volume (TV) is the amount of air that moves in/out of the lungs with each breath.The amount of air that can be inspired forcibly beyond the tidal volume (2100-3200 mL) is called the inspiratory reserve volume (IRV).

The expiratory reserve volume (ERV) is the amount of air – about 1200 mL – that can be evacuated from the lungs after a tidal expiration.

Even after the most strenuous expiration, about 1200 mL of air still remains in the lungs. This is the residual volume (RV).

Respiratory Capacities (as noted above, the respiratory capacities always consist of two or more lung volumes)

The inspiratory capacity (IC) is the total amount of air that can be inspired after a tidalexpiration. It is the sum of the tidal volume and the inspiratory reserve volume.

The functional residual capacity (FRC) is the combined residual and expiratory reserve volumes and represents the total amount of air remaining in the lungs after a tidal expiration. Vital capacity (VC) is the total amount of exchangeable air. It is the sum of the tidal,inspiratory reserve, and expiratory reserve volumes. We will gauge VC as the maximum amount of air a person exhales after filling his/her lungs to the maximum amount possible. In healthy young men, VC is approximately 4800 mL.

The total lung capacity (TLC) is the sum of all lung volumes and is normally around 6000 mL in males. Lung volumes and capacities tend to be smaller in women than in men because of women's smaller size.Pharmacist should be familiar with the type of spirometer they are using. subject should be informed about spirometry to aid adequate preparation for the test and

- **VC** = TV + IRV + ERV = 500 + 3000 + 1100 = **4600 mL**
- **TLC** = VC + RV = 4600 + 1200 = **5800 mL**
- **FVC(**

Forced Vital Capacity

) = 4600 mL

- **FEV_1**=3680mL

- **FEV_1/FVC** = 3680 / 4600 = **0.8 (or 80%)**

FEV_1/FVC	**Interpretation**
≥ 0.8	Normal
< 0.7	Obstructive (e.g., asthma, COPD)

to enable the subject togive informed consent. Many variables may affect the test - before it, the subject should avoid

- Smoking for 24 hours.
- Drinking alcohol for at least four hours.
- Vigorous exercise for at least 30 minutes.
- Wearing any tight clothing.
- Eating a large meal for at least two hours.
- Taking short-acting bronchodilators for four hours.
- Taking long-acting beta-2-agonist inhalers for 12 hours.

Procedure

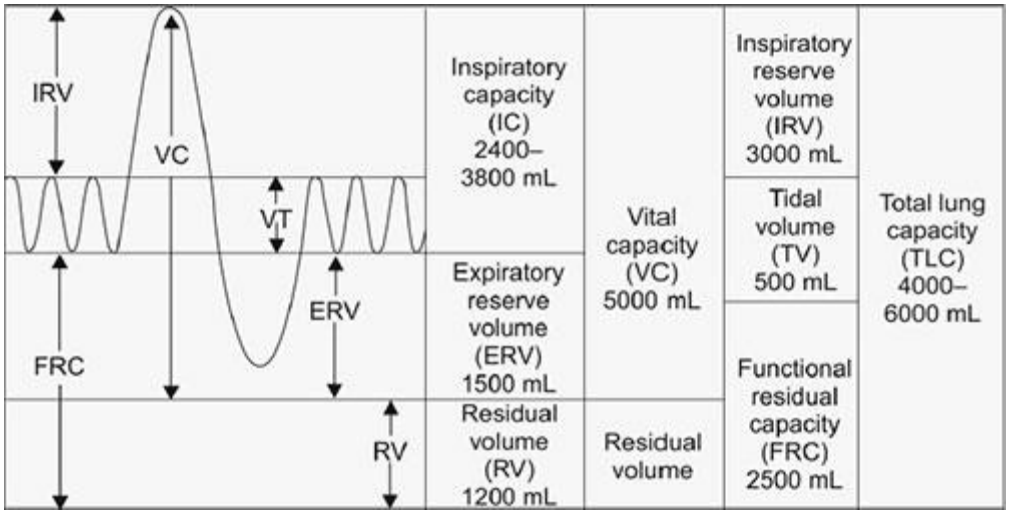
Spirometer is fill up with water and adjust zero. The subject should be seated in a chair with arms;

- Two relaxed measurements of vital capacity should be performed first, (the subject should use nose clips for this procedure to prevent air leakage from the nose), followed by three forced vital capacity measurements;
- A large breath to full inspiration is taken through mouth.
- The mouthpiece is placed into the subject mouth and the subject is asked to place his or her lips and teeth around the mouthpiece to form a tight seal.
- For the relaxed VC, the subject breathes out at a comfortable speed, but for the FVC the subject should breathe out hard and quickly until all air is expelled.

- The FVC should take 6s, but in some subjects with obstructive breathing patterns it can takeup to 15s.
- At least 30s should be left between blows (exhalations using the spirometer) to enable the subject to recover.
- A minimum of three and a maximum of eight blows should be attempted at any one time.

It is vital that subject inhale completely, to total lung capacity, and continue to exhale until they have fully emptied their lungs (to residual volume) so that a low vital capacity is not recorded due to poor effort.Calculated by using formulae.

Report:



EXPT No: 12

DATE:

STUDY OF VARIOS SYSTEM

HUMAN DIGESTIVE SYSTEM

The human digestive system consists of the gastrointestinal tract plus the accessory organs of digestion **(the tongue, salivary glands, pancreas, liver, and gallbladder**). In this system, the process of digestion has many stages, the first of which starts in the mouth. Digestion involves the breakdown of food into smaller and smaller components, until theycan be absorbed and assimilated into the body.

Chewing, in which food is mixed with saliva begins the process of digestion. This produces a bolus which can be swallowed down the esophagus and into the stomach. Here it ismixed with gastric juice until it passes into the duodenum where it is mixed with a number of enzymes produced by pancreas activation. Saliva also containsa catalytic enzyme called amylase which starts to act on food in the mouth. Another digestive enzyme called lingual lipase is secreted by some of the lingual papillae on the tongue and also from serous glands in the main salivary glands. Digestion is helped by the mastication of food by the teeth and also by the muscular actions of peristalsis and segmentation contractions. Gastric juice in the stomach is essential for the continuation of digestion as is the production of mucus in the stomach.

Peristalsis is the rhythmic contraction of muscles that begins in the esophagus and continues along the wall of the stomach and the rest of the gastrointestinal tract. This initially results in the production of chyme which when fully broken down in the small intestine is absorbedas chyle into the lymphatic system. Most of the digestion of food takes place in the small intestine. Water and some minerals are reabsorbed back into the blood in the colonof the large intestine. The waste products of digestion (feces) are defecated from the anusvia the rectum.

HUMAN RESPIRATORY SYSTEM

The respiratory system (also respiratory apparatus, ventilatory system) is a biological system consisting of specific organs and structure used for gas exchange inanimals and plants. The anatomy and physiology that make this happen varies greatly,depending on the size of the organism, the environment in which it lives and its evolutionary history. In land animals the respiratory surface is internalized as linings ofthe lungs. Gas exchange in the lungs occurs in millions of small air sacs called alveoli in mammals and reptiles, but atria in birds. These microscopic air sacs have a very rich blood supply, thus bringing the air into close contact with the blood. These air sacs communicate with the external environment via a system of airways, or hollow tubes, of which the largest is the trachea, which branches in the middle of the chest into the two main bronchi. These enter the lungs where they branch into progressively narrower secondary and tertiary bronchi that branch into numerous smaller tubes, the bronchioles. In birds the bronchioles are termed parabronchi. It is the bronchioles, or parabronchi that generally open into the microscopic alveoli in mammals and atria in birds. Air has to be pumped from the environment into the alveoli or atria by the process of breathing which involves the musclesof respiration.

In most fish, and a number of other aquatic animals (both vertebrates and invertebrates) the respiratory system consists of gills, which are either partially or completely external organs, bathed in the watery environment. This water flows over the gills by a variety of active or passive means. Gas exchange takes place in the gills which consist of thin or veryflat filaments and lammelae which expose a very large surface area of highly vascularized tissue to the water.

THE CARDIOVASCULAR SYSTEM

The cardiovascular system consists of the heart, blood vessels, and blood. Its primary function is to transport nutrients and oxygen-rich blood to all parts of the body and to carry deoxygenated blood back to the lungs.

The cardiovascular system is the system responsible for delivering blood to different parts of the body. It consists of the following organs and tissues

The heart: A muscular pump that forces blood around the body.

- **A closed system of blood vessels:** These vessels include:
    - **Arteries:** Vessels that carry blood away from the heart.
    - **Veins:** Vessels that bring blood back to the heart.
    - **Capillaries:** Tiny vessels that branch off from arteries to deliver blood to all body tissue

STRUCTURE OF THE HEART

The heart consists of four distinct chambers: two upper chambers called "atria" and two lower chambers called "ventricles." A wall or "septum" separates the atria and ventricles. Valves control the flow of blood within the different chambers.

Blood follows the following pathway through the heart:

1. Blood lacking oxygen returns from the body and enters the right atrium (upper right chamber) via the inferior vena cava and superior vena cava veins.
2. Blood flows through the tricuspid valve and enters the right ventricle (lower right chamber).
3. The right ventricle pumps blood through the pulmonary valve and out of the heart via the main pulmonary artery.
4. The blood then flows through the left and right pulmonary arteries into the lungs. Here, the process of breathing draws oxygen into the blood and removes carbon dioxide. As a result, the blood is now rich in oxygen.
5. The blood returns to the heart and flows into the left atrium (upper left chamber) via four pulmonary veins.
6. Blood flows through the mitral valve and enters the left ventricle (lower left chamber).
7. The left ventricle pumps the blood through the aortic valve into a large artery called the "aorta." This artery delivers blood to the rest of the body.

HUMAN URINARY SYSTEM

The urinary system consists of the kidneys, ureters, urinary bladder, and urethra. The kidneys filter the blood to remove wastes and produce urine. The ureters, urinary bladder, and urethra together form the urinary tract, which acts as a plumbing system to drain urine from the kidneys, store it, and then release it during urination. Besides filtering and eliminating wastes from the body, the urinary system also maintains the homeostasis of water, ions, pH, blood pressure, calcium.

The kidneys are a pair of bean-shaped organs found along the posterior wall of the abdominalcavity. The left kidney is located slightly higher than the right kidney because the right side of the liver is much larger than the left side. The kidneys, unlike the other organs of the abdominal cavity, are located posterior to the peritoneum and touch the muscles of the back. The kidneys are surrounded by a layer of adipose that holds them in place and protects them from physical damage. The kidneys filter metabolic wastes, excess ions, and chemicals from the blood to form urine.

The ureters are a pair of tubes that carry urine from the kidneys to the urinary bladder. The ureters are about 10 to 12 inches long and run on the left and right sides of the body parallelto the vertebral column.

Gravity and peristalsis of smooth muscle tissue in the walls of the ureters move urine toward the urinary bladder. The ends of the ureters extend slightly into the urinary bladder and are sealed at the point of entry to the bladder by the ureterovesical valves. These valves prevent urine from flowing back towards the kidneys. The urinary bladder is a sac-like hollow organ used for the storage of urine.

The urinary bladder is located along the body‘s midline at the inferior end of the pelvis. Urineentering the urinary bladder from the ureters slowly fills the hollow space of the bladder and stretches its elastic walls. The walls of the bladder allow it to stretch to hold anywhere from 600 to 800 milliliters of urine. The urethra is the tube through which urine passes from the bladder to the exterior of the body.

The female urethra is around 2 inches long and ends inferior to the clitoris and superior to the vaginal opening. In males, the urethra is around 8 to 10 inches long and ends at the tip of the penis.

The urethra is also an organ of the male reproductive system as it carries sperm out of the body through the penis. The flow of urine through the urethra is controlled by the

internal andexternal urethral sphincter muscles. The internal urethral sphincter is made of smooth muscle and opens involuntarily when the bladder reaches a certain set level of distention. The opening of the internal sphincter results in the sensation of needing to urinate. The external urethral sphincter is made of skeletal muscle and may be opened to allow urine to pass through the urethra or may be held closed to delay urination.

HUMAN REPRODUCTIVE SYSTEM

The reproductive system or genital system is a system of sex organs withinan organism which work together for the purpose of sexual reproduction. Many non-living substances such as fluids, hormones, and pheromones are also important accessories to the reproductive system. Unlike most organ systems, the sexes of differentiated species oftenhave significant differences. These differences allow for a combination of genetic material between two individuals, which allows for the possibility of greater genetic fitness of

the offspring. male reproductive system is a series of organs located outside of the body and around the pelvic region of a male that contribute towards the reproduction process. The primary direct function of the male reproductive system is to provide the male sperm for fertilization of the ovum.

The major reproductive organs of the male can be grouped into three categories. The first

category is sperm production and storage. Production takes place in the testes which are housed in the temperature regulating scrotum, immature sperm then travel tothe epididymis for development and storage. The second category are the ejaculatory fluid producing glands which include the seminal vesicles, prostate, and the vas deferens. The finalcategory are those used for copulation, and deposition of the spermatozoa (sperm) within the female, these include the penis, urethra, vas deferens, and Cowper's gland.

Major secondary sexual characteristics includes: larger, more muscular stature, deepened voice, facial and body hair, broad shoulders, and development of an adam's apple. Animportant sexual hormone of males is androgen, and particularly testosterone.

The testes release a hormone that controls the development of sperm. This hormone is also responsible for the development of physical characteristics in men such as facial hair and a deep voice.

The human female reproductive system is a series of organs primarily located inside of the body and around the pelvic region of a female that contribute towards the reproductive process. The human female reproductive system contains three main parts: the vulva, which leads to the vagina, the vaginal opening, to the uterus;

the uterus, which holds the developing fetus; and the ovaries, which produce the female's ova. The breasts are involved during the parenting stage of reproduction, but

in most classifications they are not considered to be part of the female reproductive system.

The vagina meets the outside at the vulva, which also includes the labia, clitoris and urethra; during intercourse this area is lubricated by mucus secreted by the Bartholin's glands. The vagina is attached to the uterus through the cervix, while the uterus is attached to the ovaries via the fallopian tubes. Each ovary contains hundreds of egg cells or ova (singular *ovum*).

Approximately every 28 days, the pituitary gland releases a hormone that stimulates some of the ova to develop and grow. One ovum is released and it passes through the fallopian tube into the uterus. Hormones produced by the ovaries prepare the uterus to receive the ovum andawaits the sperm for fertilization to occur. When this does not occur i.e. no sperm for fertilization, the lining of the uterus, called the endometrium, and unfertilized ova shed each cycle through the process of menstruation. If the ovum is fertilized by sperm, it attaches tothe endometrium and the fetus develops.

EXPT No: 13

DATE:

MEASUREMENT OF BODY MASS INDEX

Aim

To record the Body Mass Index (BMI) of the given subject

Requirements

1. Tape measure,
2. Scales,
3. Marker,
4. Clipboard

Principle

The body mass index (BMI) or Quetelet index is a value derived from the mass (weight) and height of an individual. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in metres.

The BMI may also be determined using a table[r or chart which displays BMI as a function of mass and height using contour lines or colours for different BMI categories, and which may use other units of measurement (converted to metric units for the calculation).

The BMI is an attempt to quantify the amount of tissue mass (muscle, fat, and bone) in an individual, and then categorize that person as underweight, normal weight, overweight, or obese based on that value. However, there is some debate about where on the BMI scale the dividing lines between categories should be placed. Commonly accepted BMI ranges are underweight: under 18.5 kg/m^2, normal weight: 18.5 to 25, overweight: 25 to 30, obese: over

30. People of Asian descent have different associations between BMI, percentage of body fat,and health risks than those of European descent, with a higher risk of type 2 diabetes and cardiovascular disease at BMIs lower than the WHO cut-off point for overweight, 25 kg/m^2, although the cutoff for observed risk varies among different Asian populations.

Procedure

1. Describe the testing protocol to the subject I am going to calculate your BMI by weighingyou and measuring your height.
2. Measure the subject height. Ensure the client removes their shoes. Ensure they stand upstraight with their heels against the wall. Record their height in metres
3. Measure the subject weight. Ensure they remove their shoes and wear minimal clothing.Record their weight in kilograms
4. Use the formula below to calculate the client's BMIMetric formulae

Metric BMI = Weight in Kilogram / (Height in Meters)^2

Report:

EXPT No: 14

DATE:

FAMILY PLANNING DEVICES

CONDOM

The condom is a form of male contraception. It also protects against sexually transmissible infections (STIs). A condom is a fine barrier which is rolled on to the penis before sex. It is used as a barrier to stop sperm and infection passing between sexual partners. It is usually made of rubber. If used correctly every time you have sex, condoms provide very good protection from pregnancy and infection. When condoms are used correctly every time a woman and a man have sex, two women out of 100 will get pregnant each year. Typically however, 15 out of 100 will get pregnant each year when using condoms as contraception. Viruses such as HIV cannot pass through an unbroken rubber condom. Some disadvantages appeared due to the usage of condoms such as not put on correctly torn by fingernails, jewellery or teeth, not enough lubrication (wetness) during sexual intercourse, wrong lubricant is used, prolonged or very vigorous sexual intercourse, penis goes soft before withdrawal, condom slips off during withdrawal, rubber loses its strength when condoms are kept in a warm place like a glovebox or hip pocket and rubber loses its strength because the condoms are past ―use by‖ date and rubber is damaged by some thrush treatments (clotrimazole is least likely to damage condoms).

COPPER-T

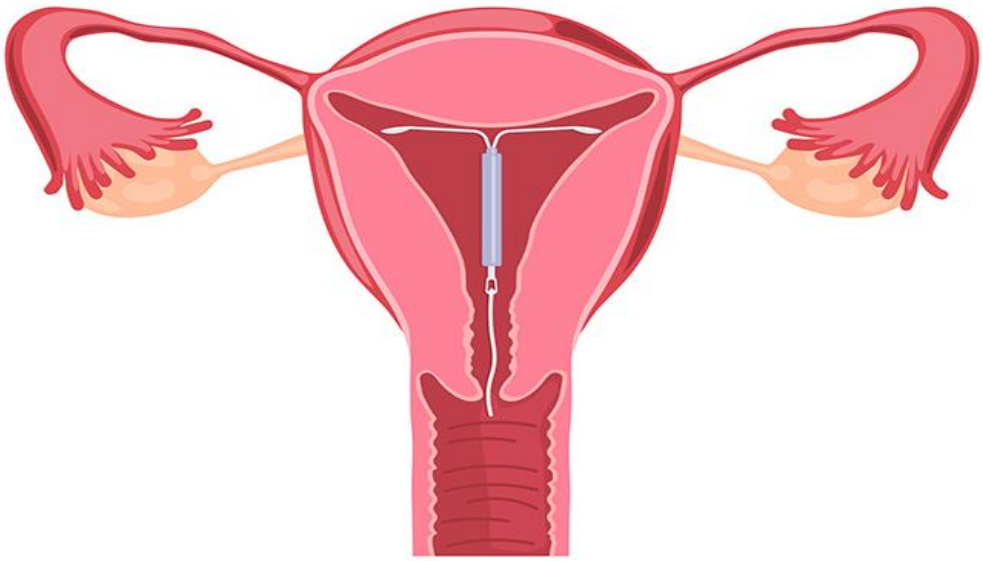
Copper T is one of the most popular and common contraceptive used in India and it is considered to be better than other contraceptives like condoms, emergency pills and birth control pills etc.-etc. They are intrauterine devices or IUDs that are very safe, low-priced and effective, they can be used to provide protection for a period of up to five years.

Copper T is a tiny device in the shape of a _T‘ which is made of plastic and draped in copper. It can fit in the palm of your hand. The shape of _T‘ helps them to rest comfortably in the opening of womb with threads hanging down. They are the best way to avoid pregnancy by stopping sperm to enter inside. Copper T prevents fertilization in two ways, the copper in the IUD kills the sperm mobility by acting as a spermicide, and it also hinders the ability of the egg to implant into the womb.

It is a preferable option for women who had a previous delivery. As it is an invasive procedure, the Copper T should be inserted with the help of an expert. The tip of the T is folded and then inserted into the patient‘s vagina. Its shape allows it to rest for years together at one place giving a better prevention than hormonal IUDs. Copper ion acts as a toxin to the sperms, it gets mixed with the uterine fluids and cervix mucus. The uterine fluid, rich in copper, acts as a spermicide which kills sperms that come in contact with it. It prevents the sperm from fertilizing an ovum after intercourse.

Copper T is a very effective device which can provide protection for almost a decade. This long lasting characteristic of Copper T makes this IUD very inexpensive and stable contraceptive. It can prove 98-100% protection from unwanted pregnancy. It doesn‘t affect your fertility and once you remove it, you can get ready to conceive again smoothly.

There are various manufacturers of Copper T in India but one should purchase from authenticsuppliers to ensure good quality and effectiveness of the device.



DIAPHRAGM

The diaphragm is a barrier method of birth control. It is moderately effective, with a one-year failure rate of around 12% with typical use. It is placed over the cervix with spermicide before sex and left in place for at least six hours after sex. Fitting by a healthcare provider is generally required.

Side effects are usually very few. Use may increase the risk of bacterial vaginosis and urinary tract infections. If left in the vagina for more than 24 hours toxic shock syndrome may occur. While use may decrease the risk of sexually transmitted infections.. There are a number of types of diaphragms with different rim and spring designs. They may be made from latex, silicone, or natural rubber. They work by blocking access to and holding spermicide near the cervix.

The diaphragm came into use around 1882. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. In the United Kingdom they cost the NHS less than 10 pounds each.

In the United States they cost about 15 to 75 USD and are the birth control method of 0.3% ofpeople.[10] These costs do not include that of spermicide.

The diaphragm does not interfere with a woman's natural cycle, therefore, no reversal or wait time is necessary, if contraception is no longer wanted or needed.

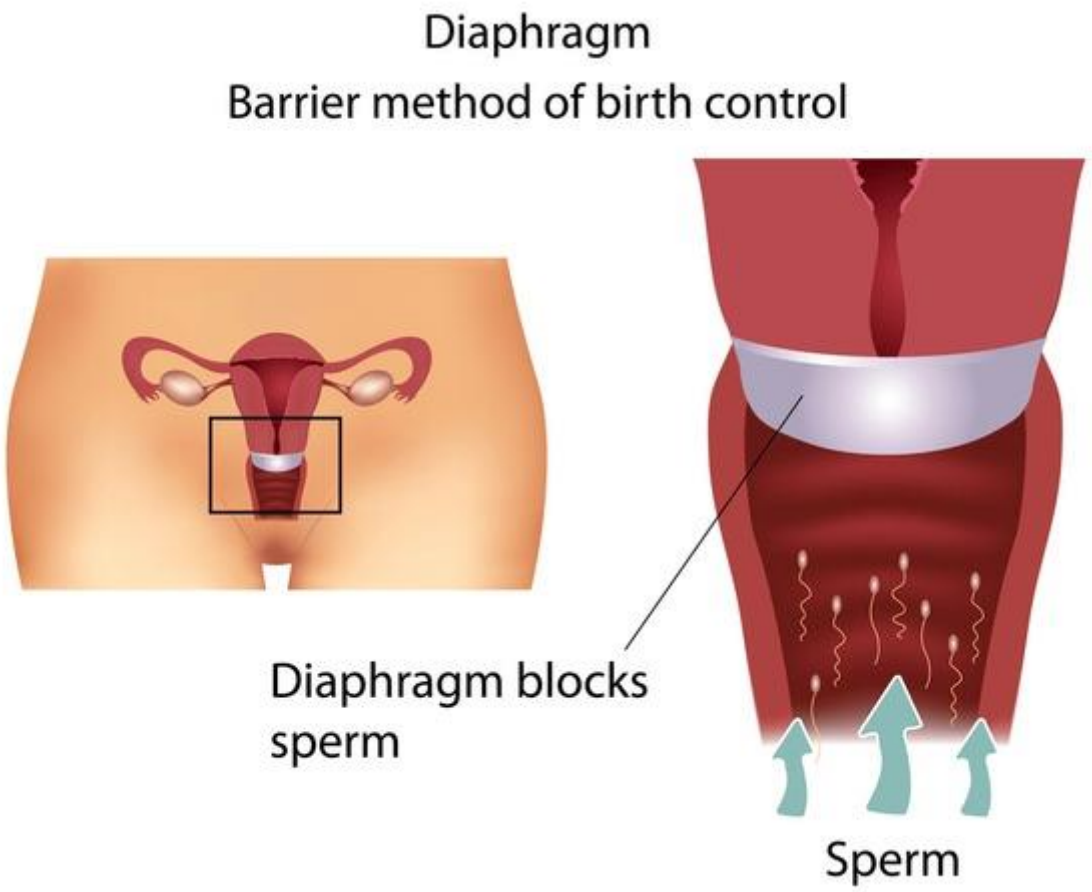
The diaphragm only has to be used during intercourse. Many women, especially those who have sex less frequently, prefer barrier contraception such as the diaphragm over methods that require some action every day.

Like all cervical barriers, diaphragms may be inserted several hours before use,

allowing uninterrupted foreplay and intercourse. Most couples find that neither partner can feel the diaphragm during intercourse.

The diaphragm is less expensive than many other methods of contraception. Women (or their partners) who are allergic to latex should not use a latex diaphragm. Diaphragms are associated with an increased risk of urinary tract infection (UTI). Urinating before inserting the diaphragm, and also after intercourse, may reduce this risk.

Toxic shock syndrome (TSS) occurs at a rate of 2.4 cases per 100,000 women using diaphragms, almost exclusively when the device is left in place longer than 24 hours.



PREGNANCY TEST

A pregnancy test attempts to determine whether or not a woman is pregnant. Indicative markers are found in blood and urine, and pregnancy tests require sampling one of these substances. The first of these markers to be discovered, human chorionic gonadotropin (hCG), was discovered in 1930 to be produced by the syncytiotrophoblast cells of the fertilised ova (eggs). While hCG is a reliable marker of pregnancy, it cannot be detected until after implantation of this results in false negatives if the test is performed during the very early stages of pregnancy. HCG can be detected via blood 8 days after fertilization of the egg, and in the urine 10 days after.

EXPT No: 15

DATE:

CELL ANALYZER

Aim

Enumerate the total counts in your blood by using cell analyzer.

Principle

Method accurately counts and sizes cells by detecting and measuring changes. The Coulter electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume(Increases of cell volume).

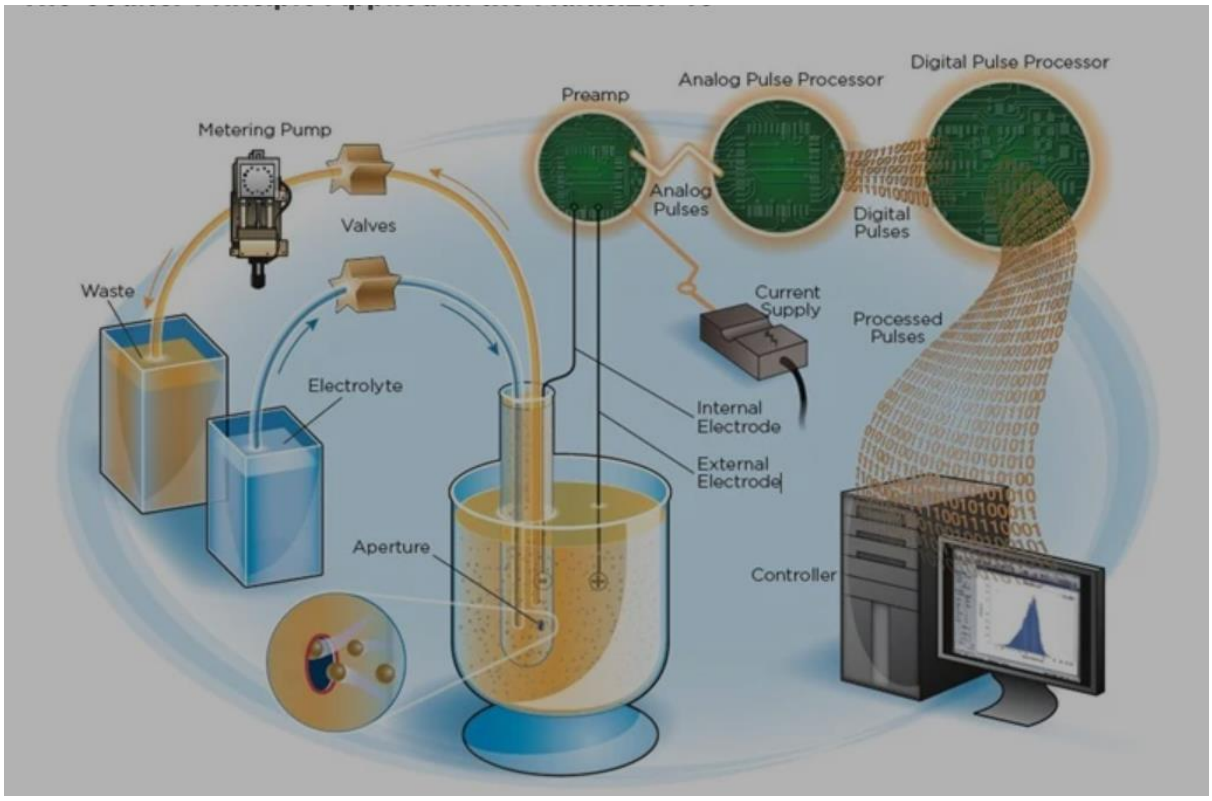
As the sample, prepared for differential analysis, streams through the flow cell these three measurements occur simultaneously on each individual white cell to classify it: - Low- frequency.

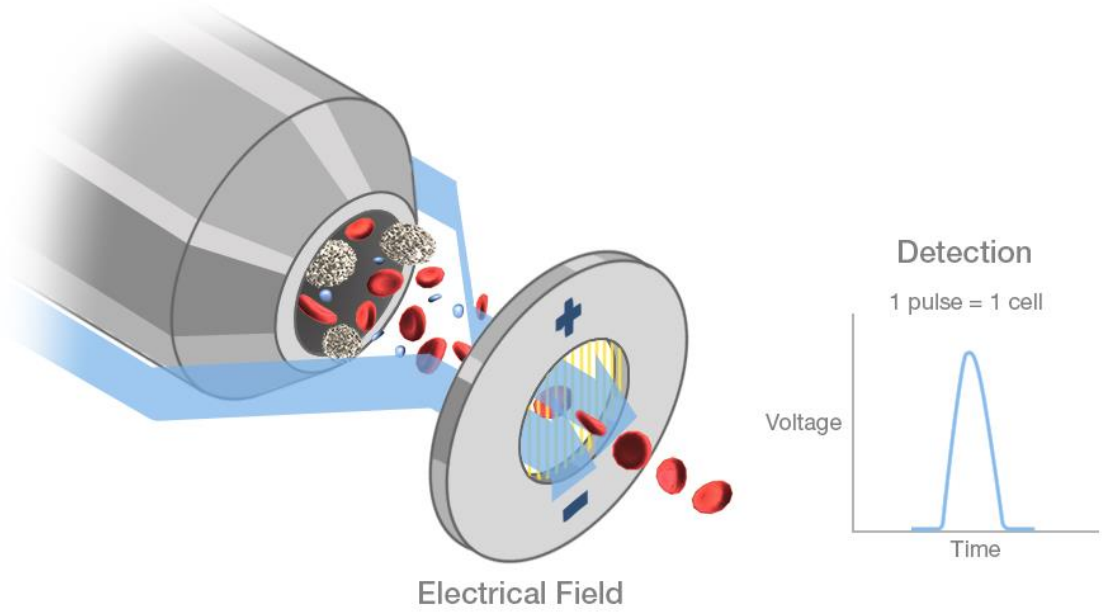
Procedure

The calibration procedure uses replicate measurements of S-CAL calibrator. The S-CAL divides the average result into the calibrator Assigned Value to give the Adjustment Factor. Then, it obtains and adjusts an instrument reading according to the Adjustment Factor.

Reagents of S-CAL consists of treated, stabilized, human erythrocytes and platelet sized components in an isotonic bacteriostatic medium. Fixed erythrocytes simulate leukocytes. Materials required – Before calibrating, assemble the following materials of S-CAL kit containing two 6-mL vials of S-CAL calibrator. Storage, handling, and stability – Sealed vials are stable through the expiration date when stored at 2-8°C (35-46°F). Open vials are stable for 1 hour. Potential biohazard – Each human donor used in preparation of this materialwas tested by an FDA-approved method for the presence of the antibodies to Human Immunodeficiency Virus (HIV-1 and HIV-2) and Hepatitis

C (HCV), as well as for hepatitis B surface antigen, and found to be negative (were not repeatedly,reactive). Handle these reagents at Biosafety Level 2 because no test method can offer complete assurance that these and other infectious agents are absent. This product contains.




Detection
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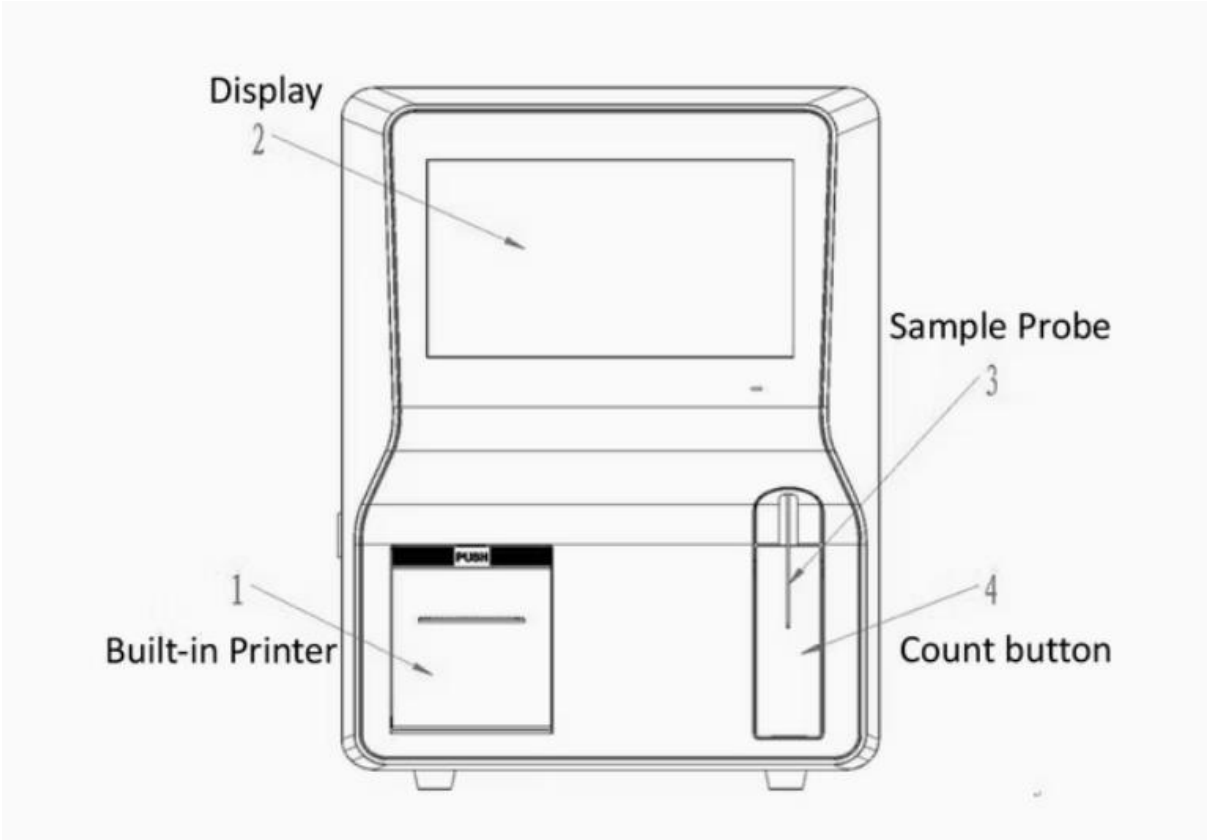
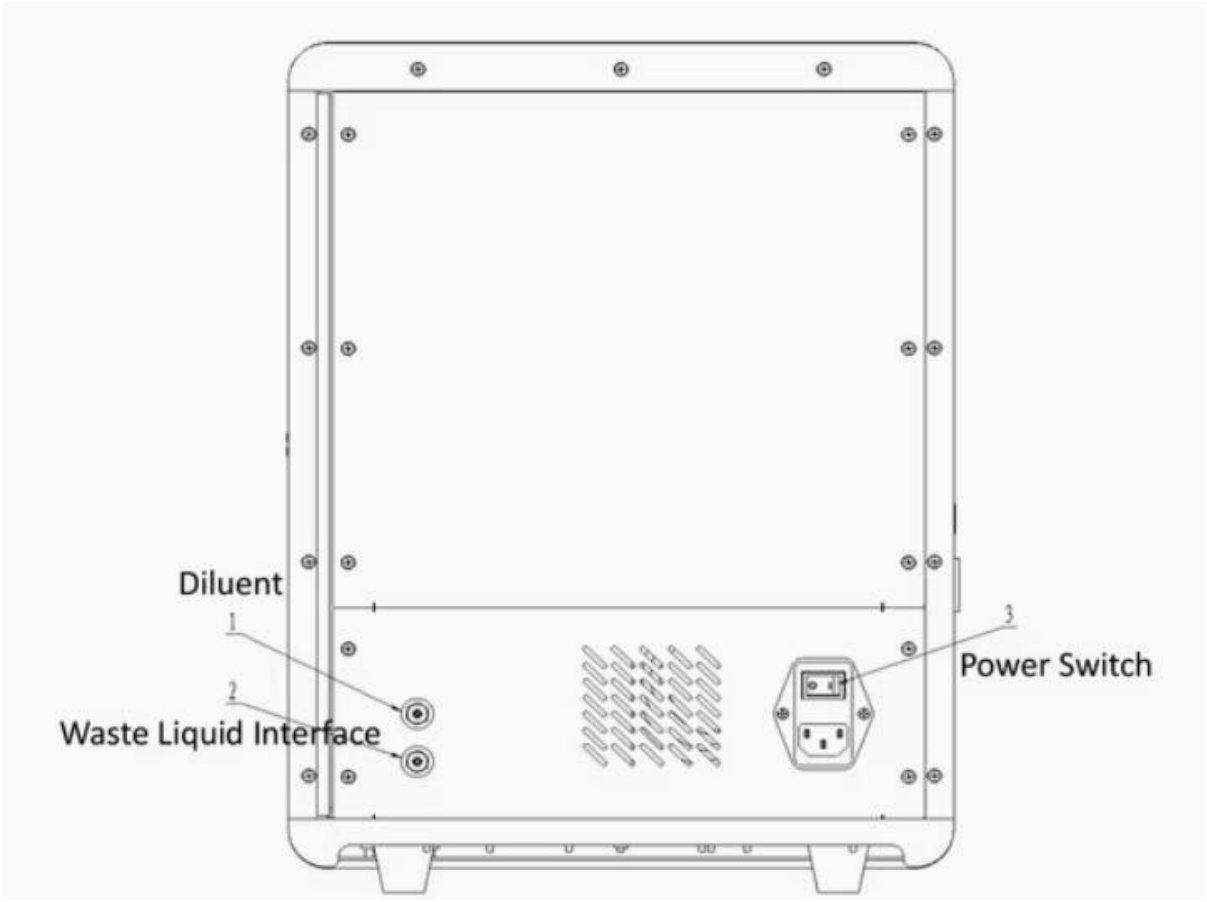
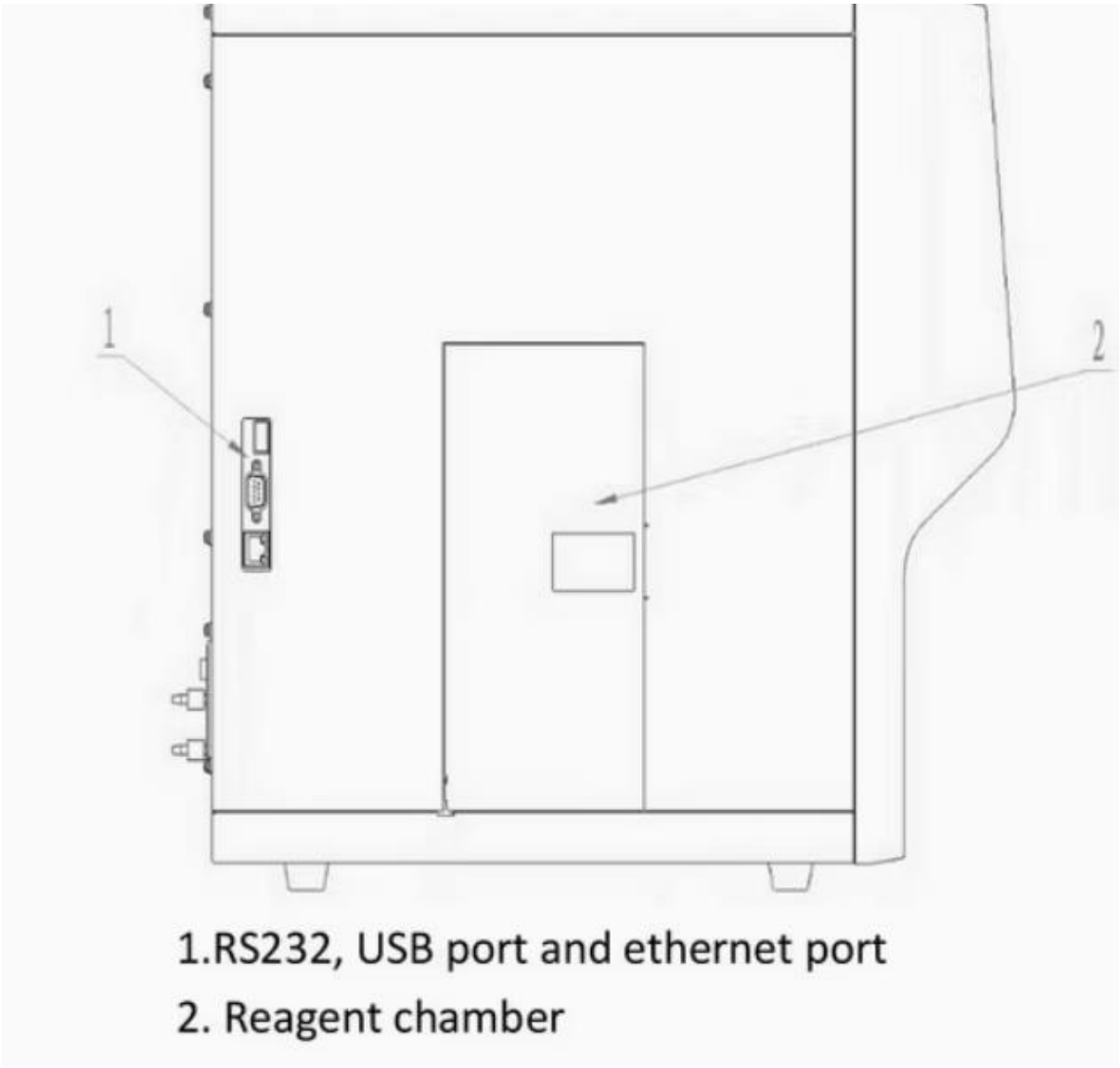
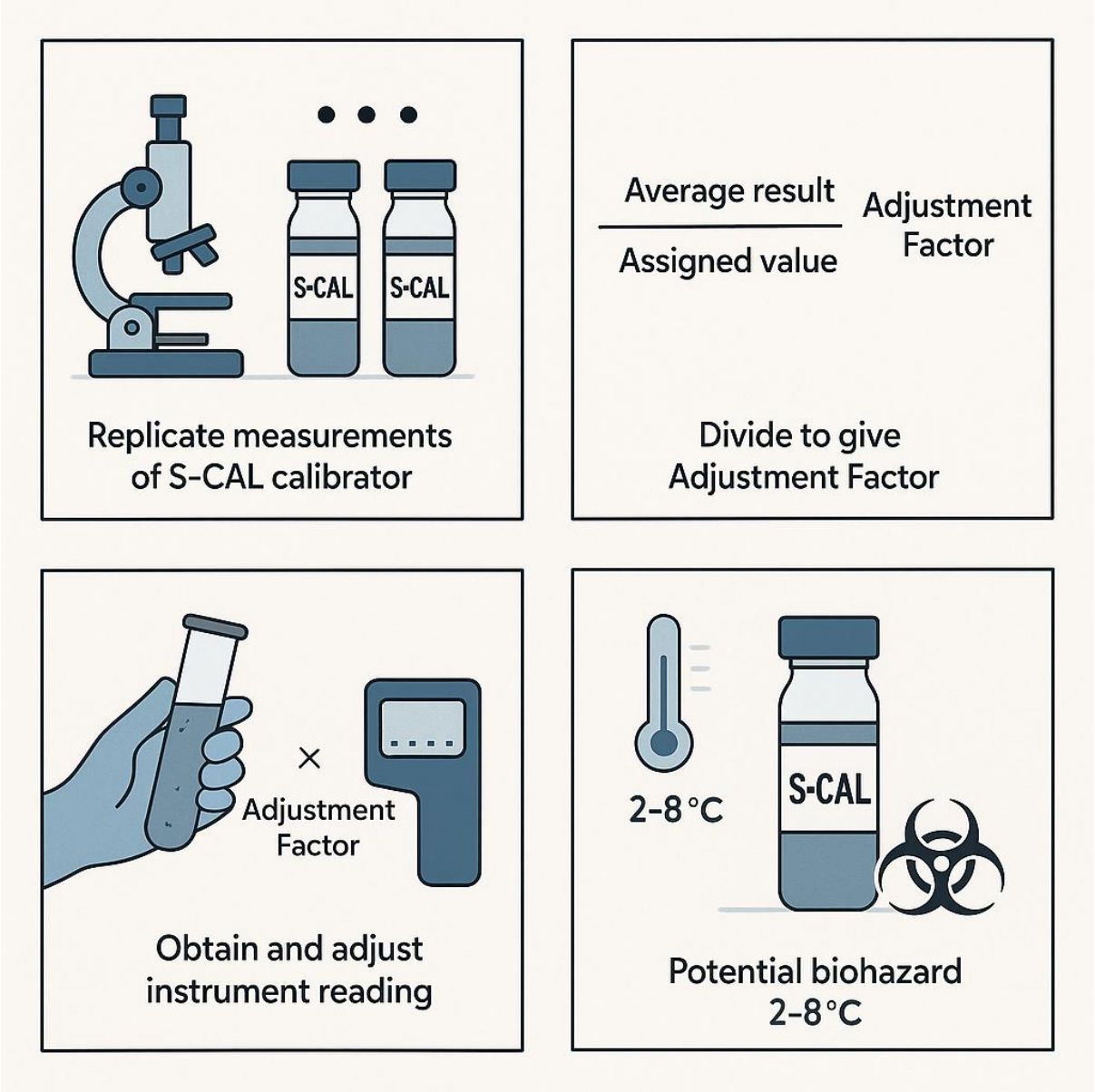
Voltage
Time
Electrical Field


Display
2
Sample Probe
3
1
Built-in Printer
4
Count button


Diluent
1
2
Waste Liquid Interface
3
Power Switch


1
2
1.RS232, USB port and ethernet port
2. Reagent chamber


S-CAL
S-CAL
Replicate measurements of S-CAL calibrator
Average result
Assigned value
Adjustment Factor
Divide to give Adjustment Factor
×
Adjustment Factor
Obtain and adjust instrument reading
2–8 °C
S-CAL
Potential biohazard 2–8 °C

Report:

EXPT No: 16

DATE:

HISTOLOGY

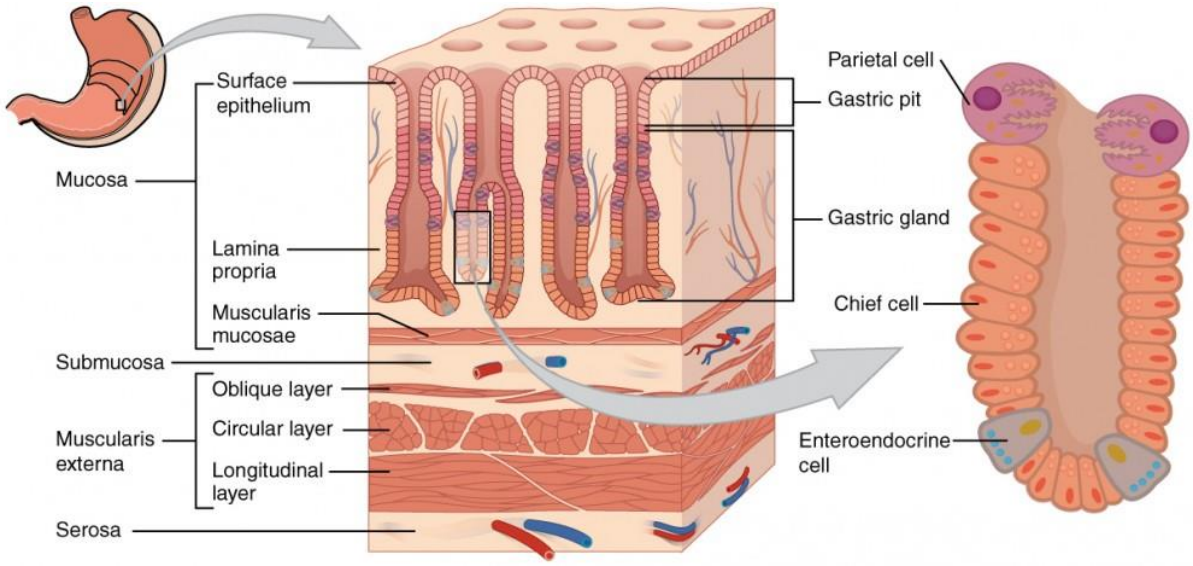
Histology or microanatomy is a study of anatomy of cells and tissues of plants and animals using microscopy. It is commonly studied using a light microscope or electron microscope, the specimen havingbeen sectioned, stained, and mounted on a microscope slide. Histologicalstudies may be conducted using tissue culture, where live animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of staining. Histology is an essential tool of biology and medicine.

STOMACH

This shows an image through the wall of the body of the stomach at low power. You should be able to identify the three major layers seen here - the mucosa, submucosa and muscularis external**.**

The mucosa is full of gastric glands and pits, and there is a prominent layer of smooth muscle - the muscularis mucosa. The contraction of this muscle helps to expel the contents of the gastric glands.

The muscularis external layer has three layers of muscle. An innner oblique layer, a middle circular and an external longitudinal layer. The contraction of these muscle layers help to break up the food mechanically.


Surface epithelium
Mucosa
Lamina propria
Muscularis mucosae
Submucosa
Oblique layer
Circular layer
Muscularis externa
Longitudinal layer
Serosa
Parietal cell
Gastric pit
Gastric gland
Chief cell
Enteroendocrine cell

ILEUM

The four layers that make up the wall of the ileum are consistent with those of the gastrointestinal tract. From the inner to the outer surface, these are

A mucous membrane, itself formed by three different layers:

A single layer of tall cells that are line the lumen of the organ. The epithelium that forms the innermost part of the mucosa has five distinct types of cells that serve different purposes, these are: enterocytes with microvilli, which digest and absorb nutrients; goblet cells, which secrete mucin, a substance that lubricates the wall of the organ; Paneth cells, most common inthe terminal part of the ileum, are only found at the bottom of the intestinal glands and releaseantimicrobial substances such as alpha defensins and lysozyme microfold cells, which takeup and transport antigens from the lumen to lymphatic cells of the lamina propria; and enteroendocrine cells, which secrete hormones.

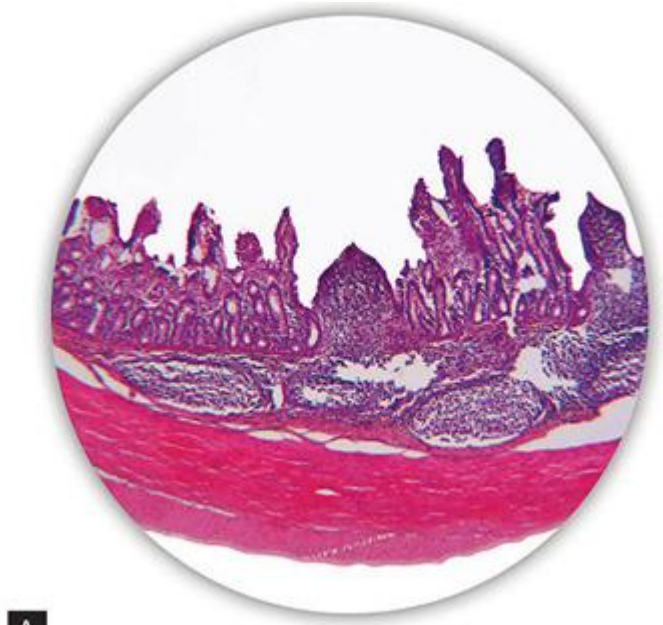
An underlying lamina propria composed of loose connective tissue and containing germinal centers and large aggregates of lymphoid tissue called Peyer's patches, which are a distinctivefeature of the ileum.

A thin layer of smooth muscle called muscularis mucosae.

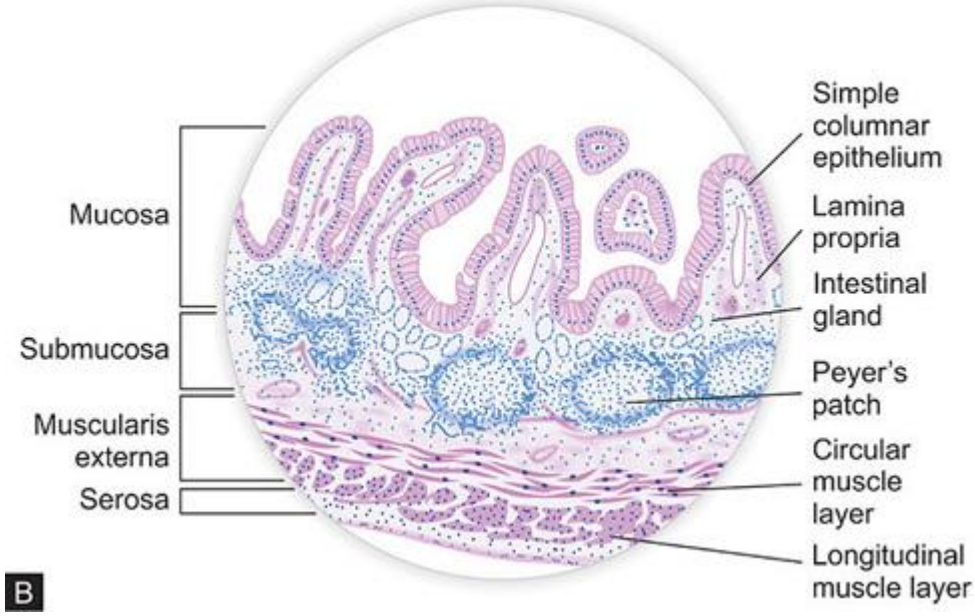
A submucosa formed by dense irregular connective tissue that carries the larger blood vessels and a nervous component called submucosal plexus, which is part of the enteric nervous system.

An external muscular layer formed by two layers of smooth muscle arranged in circular bundles in the inner layer and in longitudinal bundles in the outer layer. Between the two layers is the myenteric plexus, formed by nervous tissue and also a part of the enteric nervoussystem.

A serosa composed of mesothelium, a single layer of flat cells with varying quantities of underlying connective and adipose tissue. This layer represents the visceral peritoneumand is continuous with the mesentery.



A

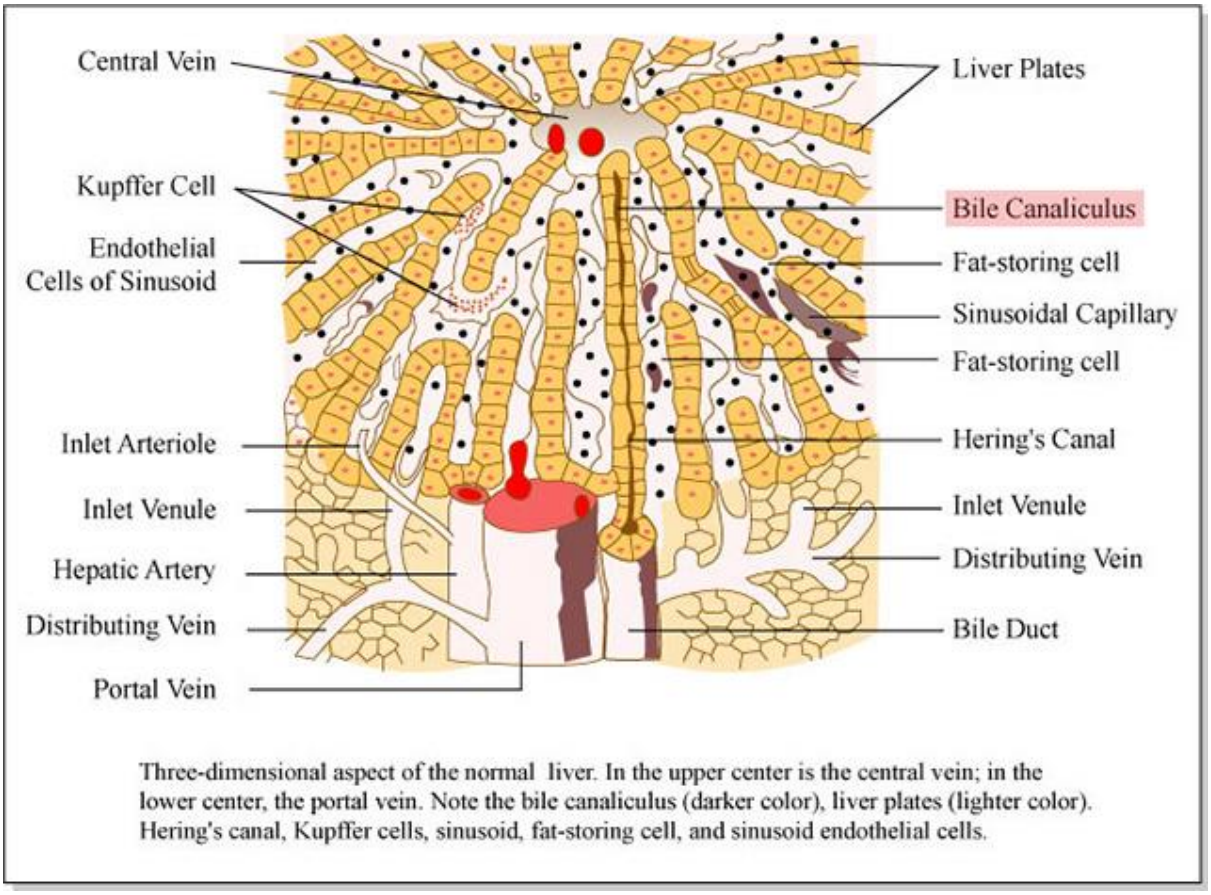

Mucosa
Submucosa
Muscularis externa
Serosa
Simple columnar epithelium
Lamina propria
Intestinal gland
Peyer's patch
Circular muscle layer
Longitudinal muscle layer

B

LIVER

Sheets of connective tissue divide the liver into thousands of small units called lobules.A lobule is roughly hexagonal in shape, with portal triads at the vertices and a central vein in the middle. The lobule is the structural unit of the liver and rather easy to observe. In contrast,the hepatic acinus is more difficult to visualize, but represents a unit that is of more relevance to hepatic function because it is oriented around the afferent vascular system. The parenchymal cells of the liver are hepatocytes. These polygonal cells are joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes. The ultrastructure appearance of hepatocytes reflects their function as metabolic superstars, with abundant rough and smooth endoplasmic reticulum, and Golgi membranes. Glycogen Hepatocytes make contact with blood in sinusoids, which are distensible vascular channels lined with highly fenestrated endothelial cells and populated with phagocytic Kupffer cells. The space between endothelium and hepatocytes is called the Spaceof Disse which collects lymph for delivery to lymphatic capillaries. granules and vesicles containing very low density lipoproteins are readily observed.

The hepatic duct is continuous with the common bile duct, which delivers bile into the duodenum. In most species, bile is diverted through the cystic duct into the gall bladder. The columnar epithelium of the gall bladder is devoted largely to absorption of water and electrolytes.



Three-dimensional aspect of the normal liver. In the upper center is the central vein; in the lower center, the portal vein. Note the bile canaliculus (darker color), liver plates (lighter color). Hering's canal, Kupffer cells, sinusoid, fat-storing cell, and sinusoid endothelial cells.

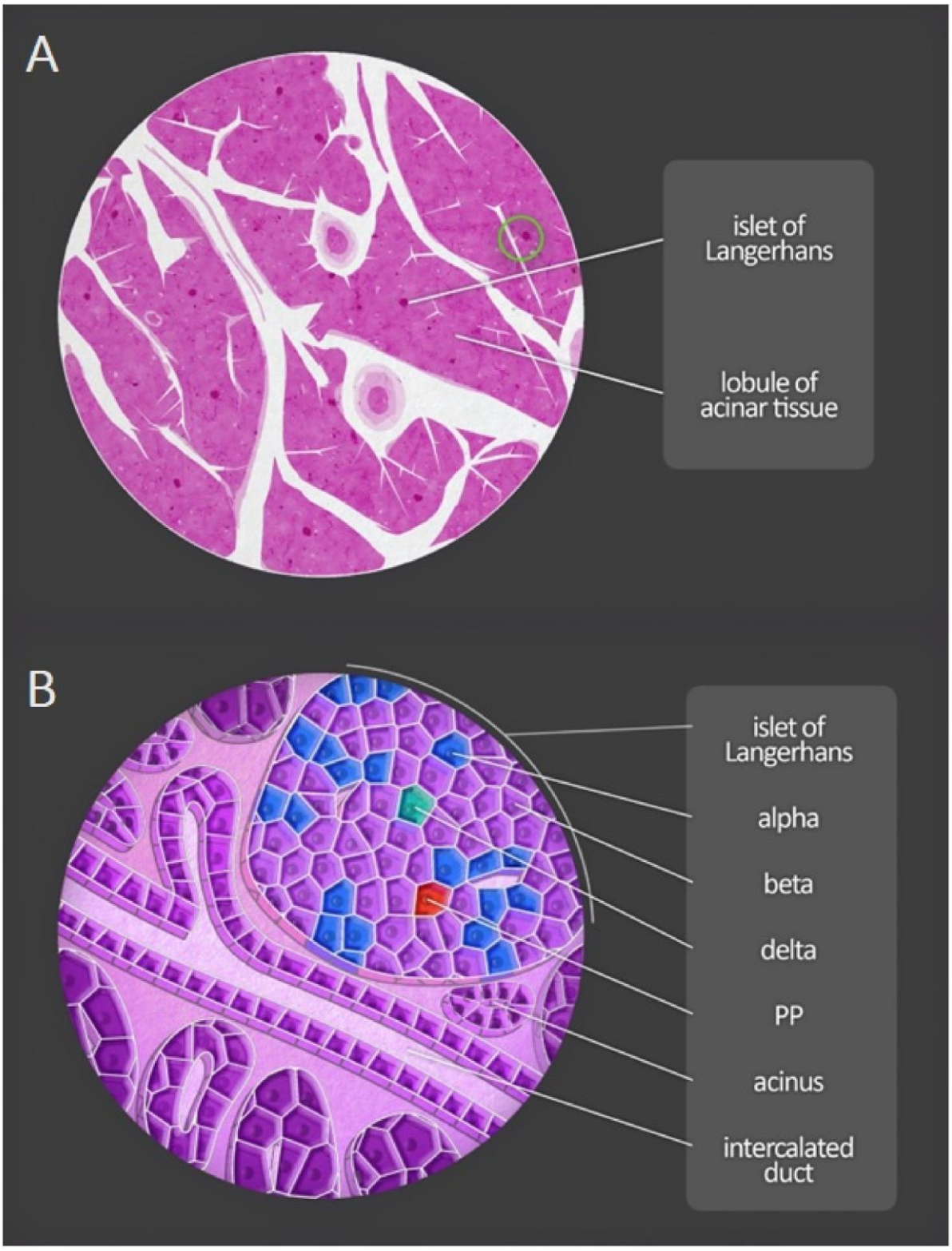
PANCREAS

The exocrine part of the pancreas has closely packed **serous acini**, similar to those of the digestive glands. It secretes an enzyme rich alkaline fluid into the duodenum via the pancreatic duct. The alkaline pH is due to the presence of bicarbonate ions, and helps to neutralise the acid chyme from the stomach, as it enters the duodenum. The enzymes digest proteins, carbohydrates, lipids and nucleic acids. These enzymes include: trypsin and chymotrypsin (secreted as inactive precursors, and activated by the action of enterokinase, an enzyme secreted by the duodenal mucosa). An enzyme called CCK stimulates the release of these enzymes, from stored granules in the secretory cells of the acini. Secretin (from neuroendocrine cells in the small intestine) stimulate the release of watery alkaline secretions.The endocrine part of the pancreas, consists of isolated islands of lighter staining cells called islets of Langerhans. The secretions of the acini empty into ducts lined with a simple low cuboidal epithelium, which becomes stratified cuboidal in the larger ducts.

The islets of Langerhans are clumps of secretory cells (up to around 3000) supported by reticulin fibres, and containing numerous fenestrated capillaries. There is a delicate capsule around each islet. They are paler than the surrounding exocrine cells, as they have less rER. These islets *do not have an acinar* organisation.

The islet cells are indistinguishable from each other in sections, but in fact three secretory cells types are present:

Alpha - Secrete
Glucagon,Beta - Secrete
Insulin
Delta - Secrete Somatostatin

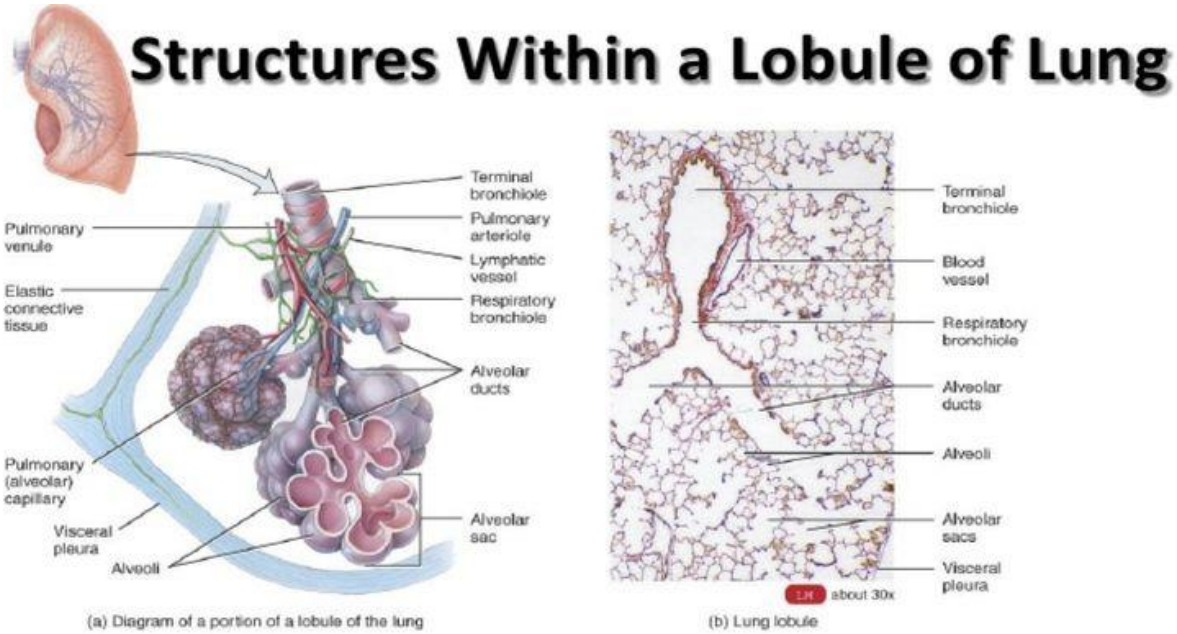

A
islet of Langerhans
lobule of acinar tissue
B
islet of Langerhans
alpha
beta
delta
PP
acinus
intercalated duct

LUNGS

The principle function of the lung is to provide oxygen to the blood and in exchange rid the blood from carbon dioxide. Inhaled air passes through the trachea that branch into two bronchi, each supplying one of the two lobes of lung. In the lung, each bronchus branches into narrower bronchi that eventually terminate in *bronchioles*. The wall of bronchioles consist of ciliated cuboidal epithelium and a thin layer of smooth muscle. As opposed to upper parts of the respiratory tract, it lacks hyaline cartilage. The main cell types of the *bronchioles* are similar to the ones in the bronchi namely, basal cells, neuroendocrine cells, *ciliated cells,* serous cells, Clara cells and goblet cells. Goblet and ciliated cellsdecrease in number as one approaches the terminal bronchioles, whereas the number of Clara cells increases proportionally. The Clara cells have a secretory function and represent the main progenitor cell after bronchiolar injury. They are columnar to cuboidal in shape and project above the ciliated cells into the airway lumen.

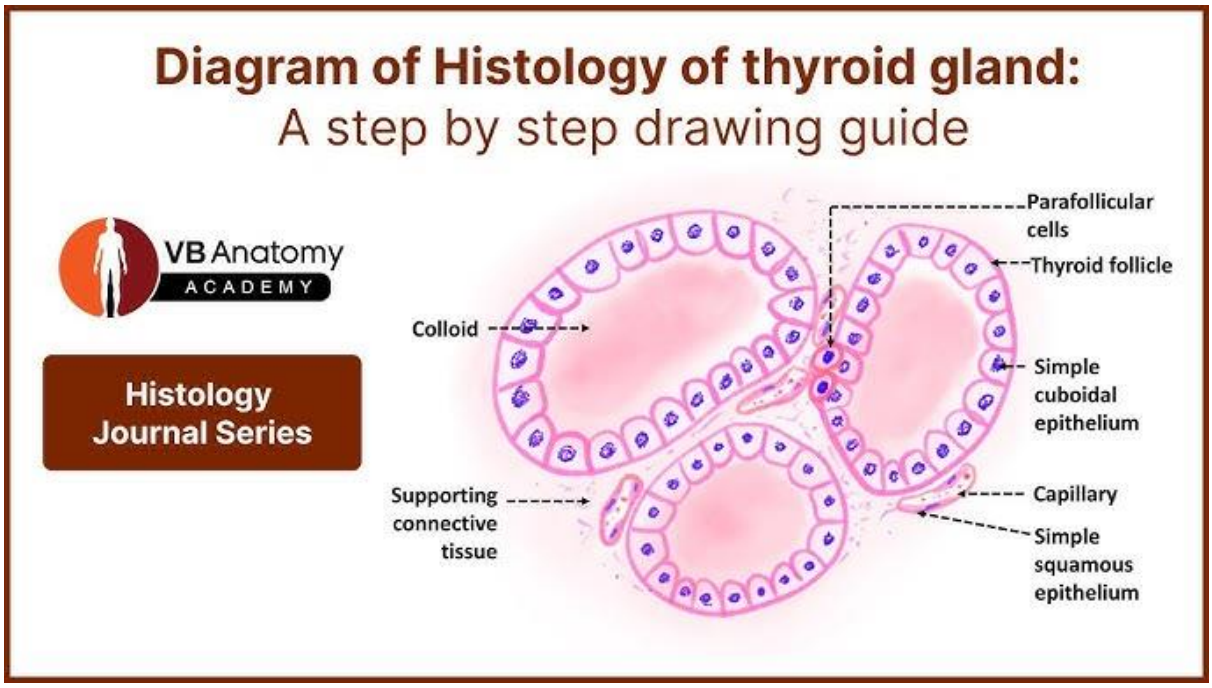
The pulmonary *alveoli* are demarcated by septa composed of a continuous layer of epithelial cells overlying a thin interstitium. Two morphologically distinct cells - type I and type II pneumocytes (alveolar cells) line the alveoli. Alveolar macrophages are also present on the epithelial surface. The interstitium contains capillaries involved in gas exchange, as well as connective tissue and a variety of cells involved in alveolar shape and defense.

Type 1 pneumocytes cover approximately 95% of the alveolar septal surface. The nucleus is small and is covered by a thin rim of cytoplasm. The rest of the cytoplasm extends as a broad sheet over the alveolar surface. Type I cells are involved in gas exchange between the alveoli and blood. Adjacent type 1 pneumocytes are joined to one another by tight junctions that provide a barrier against the diffusion of water soluble substances into the alveolar airspace. Type 2 pneumocytes are typically located near the alveolar intersections and have a granular cytoplasm. Type 2 pneumocytes have several important functions, including the productionof surfactant responsible for modifying alveolar surface tension. These cells also have the ability to be mitotically active and differentiate into type 1 cells, and synthesize substances such as fibronectin and α-1-antitrypsin that are involved in alveolar structure and defense, and enhancement of *macrophage* function.


Structures Within a Lobule of Lung
Terminal bronchiole
Pulmonary arteriole
Lymphatic vessel
Respiratory bronchiole
Alveolar ducts
Alveolar sac
Alveoli
Pulmonary venule
Elastic connective tissue
Pulmonary (alveolar) capillary
Visceral pleura
(a) Diagram of a portion of a lobule of the lung
Terminal bronchiole
Blood vessel
Respiratory bronchiole
Alveolar ducts
Alveoli
Alveolar sacs
Visceral pleura
about 30x
(b) Lung lobule

THYROID GLAND

The thyroid has a characteristic appearance under H&E stain. Most noticeable are the follicles, composed of a ring of cuboidal epithelial cells called principal cells bounded by a basement membrane and surrounding a lumen filled with pink-staining colloid that consists primarily of thyroglobulin. Also visible in this slide are a few C-cells, or parafollicular cells, scattered in the spaces between follicles. C-cells secrete calcitonin, which serves as a fine control for calcium homeostasis.



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RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

FIRST YEAR B.PHARM (II-SEMESTER)

SUBJECT: PHARMACEUTIAL ORGANIC CHEMISTRY-I (BP208P)

PREPARED BY
Dr.C.BUVANA M. Pharm,PhD.,
Professor
Department of Pharmaceutical Chemistry

CONTENTS

EXPT.NO: 01

DATE:

SYNTHESIS OF BENZANILIDE

AIM:

To prepare and submit Benzanilide from aniline with calculates its percentage yield.

REFERENCE:

1.PracticalOrganicChemistrybyMann&Saunders,4th edition,Pageno.245,246.

2.ElementaryPracticalOrganicChemistrybyArthur.I.Vogel;2nd edition;Pageno:26 9,270.

REQUIREMENTS:

Aniline, Benzoyl Chloride, 10% Sodium Hydroxide.

PRINCIPLE:

Amino compounds reacts with benzoyl chloride and gives benzoyl derivative, the reaction is known as Schotten-Baumann reaction. In this method,the benzoylation of amine is carried out with benzoyl chloride in the presence of aqueous sodium hydroxide. Benzoylation proceeds smoothly and the sparingly soluble derivative usually separates as a solid. Sodium hydroxide hydrolyses the excess of benzoyl chloride yielding sodium benzoate & sodium chloride which remain in the solution.

PROCEDURE:

1.2 ml of aniline was dissolved in 12 ml of 10% NaOH solution in a conical flask. About 1.7ml of benzoyl chloride was added. The mouth of the conical flask was corked and shakenvigorously for about 25 minutes. The precipitated benzanilide was separated, washed, driedandrecrystallisedfromalcohol.

USES:

It is used as anti atherosclerotic agent and also in the manufacture of dyes and perfumes.Benzanilide derivatives used as a fungicide and acaricide.

REPORT:

Benzanilide was prepared and submitted.

The percentage yield of the prepared Benzanilide was found to be_____%.

EXPT.NO: 02

DATE:

SYNTHESIS OF SALICYLIC ACID

AIM:

To prepare and submit the organic compound Salicylic acid from Methyl salicylate with calculates its percentage yield.

REFERENCE:

1.PracticalOrganicChemistrybyMann&Saunders,4th edition,Pageno.355-356.

2.Advanced OrganicChemistryby ArunBahlandBS Bahl;Pageno: 1236.

3.ElementaryPracticalOrganicChemistrybyArthur.I.Vogel;2nd edition;Page no:364

REQUIREMENTS:

Methylsalicylate,10%Sodiumhydroxide,Conc.Hydrochloricacid.

PRINCIPLE:

Salicylic acid is the precursor of acetyl salicylic acid. It found mainly in the willows leaves and bark. Salicylic acid possesses several useful medicinal properties. It is an antipyretics, anti- inflammatory and analgesic. Unfortunately pure salicylic acid is extremely unpleasant medicine. Two functional group in salicylic acid and irritating substance that burns the sensitive linings of the mouth,throat esophagus and stomach.

In this synthesis methyl salicylate is the starting material or precursor and salicylic acid is synthetic target. Methyl salicylate contains two functional groups (hydroxyl and ester group). Simple hydrolysis is enough to convert ester into acid and alcohol as the eliminating product. In the preparation methyl salicylate is treated with NaOH in presence of hydrochloric acid to form salicylic acid.

PROCEDURE:

Methyl salicylate (4ml) and 10% aqueous sodium hydroxide (25ml) are mixed in 250ml RBF. A small piece of porcelain is added to prevent bumping during the reflux. RBF is fitted with a reflux condenser and reflux for 45 minutes (reflux until disappearance of oily layer of ester). The contents of flask are cool and transfer into a 250ml beaker. Add conc.HCl drop by drop until the solution become acidic. Salicylic acid is liberated as a solid and filters the precipitate,washed with cold water, recrystallizes with hot water, dried in a hot air oven at 70˚C and calculate the percentage yield.

USES:

Used as antiseptic and disinfectant

Used for making dyes

For preparing aspirin, salol (phenylsalicylate),methylsalicylate

Asmedicine,it is administered as sodium salicylate in rheumatic condition.

REPORT:

Salicylicacid was prepared and submitted.

The percentage yield of salicylic acid was found to be_____%.

EXPT.NO: 03

DATE:

SYNTHESIS OF BENZAMIDE

AIM:

To prepare and submit pure sample of Benzamide.

REFERENCE:

1.PracticalOrganic ChemistrybyMann&Saunders;4th edition;Page no.119,120.

2.Advanced OrganicChemistryby ArunBahlandBS Bahl;Pageno: 1232.

3.ElementaryPracticalOrganicChemistrybyArthur.I.Vogel;2nd edition;Page no:347.

REQUIREMENTS:

Ammonia, Benzoylchloride,Beaker,conicalflask.

PRINCIPLE:

Acid amides are prepared by ammonolysis reaction of acid chloride. Acid chlorides undergo nucleophilic substitution reaction, chloride gas gets expelled as chloride ion or hydrogenchloride and its flakes is taken by some other basic group. Because of the carboxyl group,these reaction takes place much more rapidly that the corresponding nucleophilic substitution reaction of alkylhalide.

PROCEDURE:

In a conical flask 5ml of ammonia and 5ml of water was taken. To this added 1 ml of benzoylchloride and the mixture was shaken for 15 minutes. Filtered and dried the product andrecrystallisedfromwater.

USES:

Substituted benzamide has local anaesthetic properties and potent anti emetic action and alsousedas analgesics.

REPORT:

Benzamide was prepared and submitted.

The percentage yield of the prepared benzamide was found to be_____%.

EXPT.NO: 04

DATE:

SYNTHESIS OF PHENYLBENZOATE

AIM

To prepare and submit organic compound of phenyl benzoate from phenol with calculates its percentage yield.

REFERENCE

1.PracticalOrganicChemistrybyMann&Saunders,4th edition,Pageno.244,245.

2.Advanced Organic Chemistry by Arun Bahland BS Bahl ;Pageno: 1172.

REQUIREMENTS

Phenol, BenzoylChloride,10% SodiumHydroxide.

PRINCIPLE

It is an alkoxy dehalogenation reaction. The reaction between acyl halide and phenol is the best general method for the preparation of carboxylic ester. A base is frequently added to combine with the hydro-halogen when aqueous alkali is used;this is known as Scotten – Boumann reaction. Phenol undergoes benzoylation reaction, shaken well with benzoyl chloride and aqueous NaOH to give the phenyl benzoate.

PROCEDURE

1.Liquefied phenol (5ml) and aqueous NaOH (9ml) mixed in a 250ml conical flask fitted with a rubber cork and shaken well.

2.After the addition of benzoyl chloride is completed, the conical flask is corked tightly vigorously shaken for 15 to 20 minutes.

3.The disappearance of smell of benzoyl chloride and is separation of colorless solid indicates that the completion of reaction.

4.If the smell of benzoyl chloride still persists, add NaOH (5ml) shake well for 2 – 3 minutes.

5.When the reaction is complete the flask is cooled well and the product is filtered off, wash with water and dry it. The recrystallization was carried out by hot methylated spirit.

USES

It is used as variety of polyesters. Excellent starting material for the production of optical components,particularly high quality lenses for still and motion camera.

REPORT

Phenyl benzoate was prepared and submitted.

The percentage yield of the prepared phenyl benzoate was found to be ------- %.

EXPT.NO: 05

DATE:

SYNTHESIS OF BENZENEAZO-2-NAPHTHOL

AIM:

To prepare and submit Benzene azo-2-naphthol from aniline with calculates its percentage yield.

REFERENCE

1.PracticalOrganic ChemistrybyMann&Saunders,4th edition,Page no.169.

2.Advanced OrganicChemistryby ArunBahlandBS Bahl;Pageno: 1292

REQUIREMENTS

Aniline, Sodiumnitrite,2-Naphthol, SodiumhydroxideConc. Hydrochloricacid.

PRINCIPLE

Aniline ondiazotization with sodium nitrite in presence of hydrochloric acid yield benzene diazonium chloride,which then coupled with β-naphthol in alkaline medium, gives a red dye 1- Benzene azo-2-naphthol. Both diazotization & coupling reactions are required to be carried out between 0-5°C.

PROCEDURE

4ml of aniline was dissolved in a mixture of 12.8 ml conc. hydrochloric acid and 12.8 ml water and cooled this solution to 0-5°C. A solution of 3.2gm sodium nitrite dissolved in 15 ml water,was added drop wise with stirring to the cold aniline mixture. The reaction mixture was maintained at 0-5°C.When addition was completed, the solution was kept for about 5 minutes with occasional stirring.

Dissolved 6.24 gm of 2-Naphthol in 40 ml of 10% Sodium Hydroxide solution and cooled it to 0-5°C. Once the temperature condition was attained,added the diazotized solution very slowly to cold 2-naphthol solution with stirring. Thereaction mixture was allowed to stand in an ice bath for 30 minutes with occasional stirring.Filtered and separated the red crystals of Benzene Azo-2-Naphthol. Washed well with water and recrystallized from glacial acetic acid.

USES

It is used in dye industry as colouring agent

REPORT

BenzeneAzo-2-Naphtholwas prepared and submitted.

The percentage yield of BenzeneAzo-2-Naphthol was found to be

EXPT.NO: 06

DATE:

SYNTHESIS OF PICRICACID

AIM

To prepare and submit picric acid from phenol with calculates its percentage

yield.

REFERENCE

1.PracticalOrganic ChemistrybyMann&Saunders,4th edition,Page no.167.

2.Practical Medicinal Chemistry by Dr.DevalaRao ;Page no.173.

3.AdvancedOrganicChemistrybyO.P Agarwal,17thedition,Pageno.316.

REQUIREMENTS

To prepare and submit picric acid from phenol with calculates its percentage yield.

PRINCIPLE

Nitration of phenol is an example of aromatic electrophilic substitution reaction. Phenol on nitration using nitration mixture of Conc. Sulphuric acid and Conc. Nitric acid gives picric acid. The function of sulphuric acid is to furnish a strongly acid medium and to convert the nitric acid in the highly reactive nitronium ion, NO_2^+ which is the real nitrating agent, acting as the electrophile. This is carried out at low temperature because at high temperature they may be loss of reactants due to the oxidizing action of nitric acid. This reaction involves the deactivating group $-NO_2$, $-SO_3H$, the intensity of the reaction is increased by using either fuming sulphuric acid or fuming nitric acid.

PROCEDURE

4ml Conc.H_2SO_4 is taken in a china dish and 3ml phenol is added slowly with stirring.

The reaction mixture is homogenized by warming on a water bath. It is the cooled well, phenol sulfonic acid is formed

12 ml Conc. Nitric acid is taken in a 250 ml RBF and cooled well.

Phenol sulfonic acid is added in drops, with constant shaking to the nitric acid, during this mixture, heat is liberated. So the mixture is cooled well under the tap water and the temperature is maintained by such away that it does not rise above 60^{o}C. Red brown fumes were evolved.
When the reaction has subsided, the content are heated on a boiling water bath for 2 hours. The flask is cooled and about 100 ml ice water is added and the flask is kept in the ice bath.

Picric acid separates out as a yellow solid. The yellow solids are filtered off, washed with water thoroughly to remove all acids, dried with filter paper and the yield is noted and recrystallized from hot rectified spirit.

USES

It is used as a yellow dye to silk and wool. It is used to identify reducing and non reducing sugar. It reacts with reducing sugar to form maha gony red color. It is used as an antiseptic. It is used to prepare explosives.

REPORT

Picric acid was prepared and submitted.

The percentage yield of Picric acid was found to be_____

EXPT.NO: 07

DATE:

SYNTHESIS OF BENZOIC ACID

AIM

To prepare and submit the organic compound benzoic acid from Benzaldehyde with calculates its percentage yield .

REFERENCE

1.PracticalOrganic ChemistrybyMann&Saunders,4th edition,Page no.120.

2.Advanced Organic ChemistrybyArunBahlandBSBahl;Page no:1226- 1233.

REQUIREMENTSS

Benzamide,10%sodiumhydroxide, Conc.hydrochloricacid.

PRINCIPLE

Benzoic acid helps to prevent the infection caused by bacteria. It is also used to treat skin irritation and inflammation caused by burns, insect bites, and fungal infection

It is colorless crystalline solid and a simple carboxylic acid. This is produced by heating the aromatic aldehyde with potassium or sodium hydroxide. Benzoic acid is prepared by using Cannizarro reaction, discovered in 1853.

The Cannizarro reaction take place by nucleophilic addition of OH to unionized aldehyde (bearing no α hydrogen) to give the tetrahedral intermediate which expel hydride ion as a leaving group and is there by oxidized. An aldehyde molecule accepts the hydride ion in another nucleophilic addition step and is there by reduced.

PROCEDURE

A mixture of Benzaldehyde (2ml), Potassium permanganate solution (60ml), Sodium hydroxide (2g) was taken in an iodine flask. Warm the iodine flask on a water bath for 40 minutes.

Acidified with conc.HCl and add 25% sodium sulphate solution until the manganese dioxide has dissolved. The mixture is cooled well and product crystallized out. Recrystallization was carried out with hot water.

USES

It is used as expectorant, antiseptic, analgesic, food preservatives (sodiumbenzoate).

REPORT

Benzoic acid was prepared and submitted.

The percentage yield of benzoic acid was found to be_____%.

EXPT.NO: 08

DATE:

SYNTHESIS OF ASPIRIN

AIM

To prepare and submit acetyl salicylic acid(aspirin) from Salicylic acidwith calculates its percentage yield

REFERENCE

1.PracticalOrganic ChemistrybyMann&Saunders,4th edition,Page no.110- 111.

2.Comprehensive Practical Organic Chemistry by V.KAhluwaliaand R,Agarwal;

Pageno.3-4.

REQUIREMENTS

Salicylic acid, Acetic anhydride, Conc.H_2SO_4

PRINCIPLE

Phenols, unlike amines, cannot be acetylated satisfactorily in aqueous solution,acetylation proceeds readily with acetic anhydride in the presence of a little concentrated Sulphuric acid as catalyst. Salicylic acid(o-hydroxy benzoic acid) upon acetylation yields acetyl salicylic acd(aspirin). Aspirin decomposes when heated and does not possess atrue, clearly defined melting point(decomposition points ranging from 128˚C to 135˚C).

PROCEDURE

In a 250ml conical flask take 5g salicylic acid,7ml acetic anhydride & 5-6 drops of conc.sulphuric acid. Shake the mixture thoroughly. Warm the reaction mixture with constant stirring on a waterbath maintained at 50-60 ˚C for about 15 minutes. Cool & then pour it in 100ml cold water taken in a 250ml beaker with stirring. Filter the precipitated crude product. Recrystallize from 50% acetic acid (a mixture of equal volume of water and acetic acid).

USES

It is used as antipyretics, anti-inflammatory and anti-platelet.

REPORT

Aspirin was prepared and submitted.

The percentage yield of Aspirin was found to be ------

INTRODUCTION TO USE OF STEREOMODELS

SL.NO	TYPE OF BOND	BOND LENGTH(A˚)
1	Csp^3 - Csp^3	1.54
2	Csp^3 - Csp^2	1.50
3	Csp^3 -Csp	1.46
4	Csp^2 - Csp^2	1.48
5	Csp^2 - Csp	1.43
6	Csp – Csp	1.38
7	Csp^3 – H	1.11
8	Csp^2 – H	1.10
9	Csp – H	1.08
10	Csp^2 = Csp^2	1.34
11	Csp ≡ Csp	1.21
12	Csp^2 ------- Csp^2	1.397
13	C = O	1.20

The various colours of balls in the atomic model 'set' represents:

Black : Carbon
Red : Oxygen
Blue : Nitrogen
Green : Phosphorous, Sulphur
White : Hydrogen

EXPT.NO:09

DATE:

STEREO MODEL OF METHANE

AIM

To construct stereo models of certain organic compounds and understand concept of bond angle,bond length etc.

MATERIALS REQUIRED

Atomic model set, Molecular atom

PROCEDURE

Constructions of stereo model of methane using ball and sticks were attempted and bond angle, bond length, bond formation in respective molecule were visualized and correlated with the values given in the standard reference.

In ball and sticks model, ball with black, red and white colour respectively represent carbon, oxygen and hydrogen respectively. Some of the concepts are discussed below:

Bond angle : in case,methane bond angle is 109˚28'

Bond length : C-H bond length is 1.11A˚

Type of hybridization : sp^3

Bond formation : 4 σ bonds formed by overlapping $4sp^3$ hybrid of carbon atoms with atomic orbitals of hydrogen atom.

REPORT

Stereo models of methane is assembled and submitted.

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PRACTICAL MANUAL

FIRST YEAR B.PHARM (II-SEMESTER)

BIOCHEMISTRY- BP209P

PREPARED BY
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Department of Pharmaceutical Chemistry

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23		QUALITATIVE ANALYSIS OF URINE SAMPLE- SAMPLE NO:1			
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BUFFERS

Definition:

A solution that can resist the change in pH is termed as buffer solution. The resistance of a solution to change in hydrogen ion concentration upon the addition of small amount of acid or alkali is termed as buffer action, a solution which passes such properties is known as buffer solution. The pH of pure water is7 and the water has no buffer action.

Types of buffer:

Acidic buffer solutions: These are the solution having mixture of weak acid (CH_3COOH) and its salt (CH_3COONa).

Basic buffer solutions: These are the solution having mixture of weak base (NH_4OH) and its salt (NH_4Cl).

Buffer action:

Buffer solutions undergo only small change of pH on the addition of acid or base. The resistance of a solution to change in H^+ ion concentration upon addition of small amount of acid or alkali is termed as buffer action.

Mechanism of buffer action:

If a small quantity of 0.1N HCl is added to the buffesolution the basicitiers up the H^+ ions released by HCl.

$$HCl + H_2O \rightarrow H_3O^+ + Cl^-$$

$$CH_3COO^- + H_3O^+ \rightarrow CH_3COOH + H_2O$$

If an alkali (NaOH) is added to the buffer solution, the free acid of the buffer solution neutralizes the base added.

$$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$$

Thus the change of pH is resulted by the addition of small quantities of strong acid. The buffer action of weak base and its conjugated acid (NH_3- NH_4Cl) may be explained as: If the acid added, the NH_3 combines with H_3O^+ ions produced by reaction of HCl with H_2O.

$$HCl + H_2O \rightarrow H_3O^+ + Cl^-$$

$$NH_3 + H_3O^+ \rightarrow NH_4^+ + H_2O$$

If an alkali is added it combines with NH_4^+ ions produced by the alkali is dissociation of NH_4Cl.The NH_4^+ ions being an acid combines with the alkali which dissociates into Na^+ and OH^-.

$$NH_4^- + OH^- \rightarrow NH_3 + H_2O$$

Thus the change in PH is resisted.

BUFFER CAPACITY:

Resistance to change in P^H on the addition of an acid or alkali is resisted to as buffer action. The magnitude of the resistance of a buffer solution to P^H changes is called its buffer capacity. It may otherwise be called as buffer co-efficient, buffer index or value as given as

$$\beta = A\beta / A\,PH$$

Where, β - buffer capacity

Aβ - Small increment in g m equivalent per litre of strong base added to the buffer solution, to produce a change in P^H (A P^H)

PROPERTIES OF BUFFER:

(i) P^H of buffer solution remains constant.

(ii) P^H of solution does not change in dilution.

(iii) P^H does not change even after addition of small quantities of acid or bases.

(iv) P^H does not change on keeping for long time.

PHYSIOLOGICAL BUFFERS

Acids or Bases after their formation in the cell pass via the extracellular fluid to the site of excreation, without any change in the ECF. This is due to the efficient buffer system which is present in the fluids and erythrocytes.

Primary buffer components in plasma:

(a) Carbonic acid-bicarbonate buffer system
(b) Acid or alkali salts of Phosphoric acid- secondary buffer system in the erythrocytes
(c) Hemoglobin- protein buffer system
(d) Acid/alkali salts of phosphoric acid sytem

ROLE OF BUFFERS IN PH REGULATION

(i) Principle buffers of extra cellular fluids.
    a) Bi-carbonate buffer
    b) Phosphate buffer
    c) Protein buffer
(ii) Principle buffers of intra cellular fluids.
    Phosphate buffer
    Protein buffer
(iii) Principle buffers of erythrocytes
    Haemoglobin buffer

(i) Principle buffers of extra and intra cellular fluids

(a) **Bi- Carbonate buffer**:

It is the main buffer in blood plasma and it consist of HCO_3^- and H_2CO_3 strong bases are converted into weak acids with the rise of HCO_3^- .

The bicarbonate buffer neutralize strong acids to weak bases are convertedto weak base (A) with increase in H_2CO_3, Strong bases are converted to weak acid with rise of HCO_3^-.

$$HA + HCO_3 \rightarrow A^+ + H_2CO_3$$

$$B + H_2CO_3 \rightarrow BH^+ + HCO_3$$

(b) Phosphate buffer:

It is mainly an intracellular buffer its concentration in plasma is very low. But for this fact it would have been the most effective buffer in plasma. Because the pka value 6.8 it may be very near to physiological P^H is 7.5.

(c) Protein buffer:

The protein buffers are very important in the plasma and the intracellular fluids but the concentration is very low in ECF, lymph and intestinal fluids.

(iii) Principle buffers of erythrocytes:

In the erythrocytes, the two buffer systems consist of haemoglobin/ oxy haemoglobin and acid/ alkali potassium salts of phosphoric acid.

The buffer capacity of blood in physiological range P^H 7.0 to 7.8. Usually when the P^H of the blood goes below 6.9 or 7.8 life is in serious danger. The P^H of the blood in diabetic, coma is alleged to drop as low as about 6.8.

QUALITIES OF BUFFER:

(i) Optimizing biological activity

(ii) Increasing stability

(iii) Improving purity

(iv) Enhancing solubility

(v) Comforting the body.

EXP NO:01

DATE:

PREPARATION OF ACETATE BUFFER OF P^H 3.7

AIM:

To prepare acetate buffer P^H 3.7 as per Indian Pharmacopeia.

REFERENCE:

1) Indian pharmacopeia.(1996),Vol II, Pg No:A-145

2) Indian pharmacopeia.(2007),Vol-3,Pg No:478

3) Indian pharmacopeia.(2014), Vol IV,Pg No: 758

2) An Introduction to Practical Biochemistry,David T plummer,Pg No:52

REAGENT REQUIRED:

(i) Anhydrous sodium acetate

(ii) Glacial acetic

acid (iii) Distilled

water.

STANDARD FORMULA:

(i) Anhydrous sodium acetate-1gm

(ii) Glacial acetic acid- to adjust PH 3.7

(iii) Distilled water- 300ml

(iv) Water to make up- 1000 ml

WORKING FORMULA:

(i) Anhydrous sodium acetate-1gm

(ii) Glacial acetic acid- to adjust PH 3.7

(iii) Distilled water- 300ml

(iv) Water to make up- 1000 ml

PROCEDURE:

Dissolve 1gm of anhydrous sodium acetate in 30ml of water, adjusted the **P^H** to 3.7 with glacial acetic acid and diluted with water to 100ml. then adjusted the **P^H** to 3.7 before use.

REPORT:

EXP NO:02
DATE:

PREPARATION OF SALINE PHOSPHATE BUFFER

AIM

To prepare saline phosphate buffer of P^H of 7.4

REFERENCE :

1) Indian pharmacopeia.(1996),Vol II, Pg No:A-147

2) Indian pharmacopeia.(2007),Vol-3,Pg No:480

3) Indian pharmacopeia.(2014), Vol IV,Pg No: 761

REAGENTS REQUIRED

(i) Disodium hydrogen phosphate- 2.38gm
(ii)Potassium dihydrogen phosphate- 0.19gm
(iii) Sodium chloride-8 gm
(iv) Distilled water-1000 ml

WORKING FORMULA

(i) Disodium hydrogen phosphate- 1.19gm
(ii)Potassium dihydrogen phosphate- 0.095gm
(iii) Sodium chloride-4 gm
(iv) Distilled water-500 ml

PROCEDURE

Dissolve 1.18gm of disodium hydrogen phosphate, 0.095 gm of potassium dihydrogen phosphate and 4 gm of sodium chloride in small amount of water and make up to 500 ml with distilled water.

DISCUSSION

The phosphate buffer system enjoys the advantage of containing the dihydrogen and monohydrogen phosphate anions, one of the physiological pair found in body. The pKa value of dihydrogen phosphate anions 7.2 maintained, this is a very distinct buffer system PH of physiological fluid, phosphate also support microbial growth. But may be preservative.

REPORT:

EXP NO:03

DATE:

PREPARATION OF CITRO PHOSPHATE BUFFER OF P^H 7

AIM:

To prepare citro phosphate buffer P^H 7 as per Indian Pharmacopeia.

REFERENCE:

1) Indian pharmacopeia.(1996),Vol II, Pg No:A-146

2) Indian pharmacopeia.(2007),Vol-3,Pg No:479

3) Indian pharmacopeia.(2014), Vol IV,Pg No: 759

REAGENT REQUIRED:

a) Citric acid (2.1%)

b) Di sodium hydrogen phosphate

c) Distilled water

STANDARD FORMULA:

(i) Citric acid - 17.6 ml

(ii) Di sodium hydrogen phosphate - 82.4ml

(iii)Distilled water – make up to 100ml.

WORKING FORMULA:

(i) Citric acid - 17.6 ml

(ii) Di sodium hydrogen phosphate - 82.4ml

(iii) Distilled water – to make up to 100ml

PROCEDURE:

17.6ml of 2.1% solution of citric acid was mixed with 82.4ml of di sodium hydrogen phosphate and made up to 100ml with distilled water.

REPORT:

EXP NO:04
DATE:

PREPARATION OF CARBONATE BUFFER

AIM

To prepare carbonate buffer of P^H of 9.7

REFERENCE:

1) Indian pharmacopeia.(1996),Vol II, Pg No:A-146

2) Indian pharmacopeia.(2007),Vol-3,Pg No:479

3) Indian pharmacopeia.(2014), Vol IV,Pg No: 759

REAGENTS REQUIRED

Sodium bicarbonate – 8.4gm

Sodium carbonate – 10.6gm

Water - to make up to 500ml

WORKING FORMULA

Sodium bicarbonate – 1.68gm

Sodium carbonate – 2.12gm

Water - to make up to 100ml

PROCEDURE

Dissolve 1.68gm of sodium bicarbonate and 2.122gm of sodium carbonate in sufficient water to produce 100ml.

DISCUSSION

The principle advantages of this buffer results because of relative insolubility of most metal carbonate and because of the sensitivity of P^H to temperature changes. High temperature causes extreme P^H changes due to loss of carbon dioxide.

The buffering range in which these buffers work well is 10 – 10.8.

REPORT:

EXP NO :05
DATE :

COLORIMETRY

Many biochemical experiments involve the measurement of a compound or group of compounds present in a complex mixture probably the most widely used method for determining the concentration of biochemical compounds is colourimetrically which makes the use of the property that when light passes through a coloured liquid, some wave lengths are observed more than the other. Many compounds are not themselves coloured but can be made to absorb light in the visible region with suitable reagent.

The big advantage is that complete isolation of compound is not necessary and the constituent of a complex mixture. The depth of colour is proportional to the concentration of compound being measured, while the amount of light absorbed is proportional to the intensity of colour and hence to the concentration.

Colorimetry is a technique widely used in biochemical estimation. It involves quantitative estimation of colour. A substance estimated by corimetry, must be either coloured or more commonly, capable of forming chromogens, through interaction with suitable reagents.

BEAR-LAMBERT'S LAW

When a ray of monochromatic light of initial intensity of I passes through the solution in a transparent vessel, some light is absorbed so that the intensity of transmitted light I is less than I_0. There is some less less of light intensity from scattering by particles in the solution and reflection at the interface but mainly from absorption by the solution. The relationship between I and I_3 depends on the path length of the above absorbing medium "l" and the concentration of observed solution 'c' this factors are related in the laws of lamberts and bear.

According to Lambert's law , when a ray of monochromatic light passes through an absorbing medium its intensity decreases exponentially as the length of the absorbing medium increases.

$$I=I\times e^{-k_1 l}$$

According to Bear's law , when a ray of light passes through an absorbing medium its intensity decreases exponentially as the concentration of the absorbing medium increases.

$$I_0=I_0\times e^{-k_2 l}$$

These two laws are combined together in the Bear-Lambert Law,

$$I_0=I_0 \times e^{-k_3 l}$$

TRANSMITTANCE

The ratio of intensities is known as the transmittance (T) and this is usually expressed as percentage.

$$T = I/I_0 \times 100 = e^{-k_3 l}$$

This is not very convenient since a plot of percent transmittance against concentration gives a negative exponential wave.

LIMITATIONS OF BEAR-LAMBERT LAW

- Light must be of a narrow wavelength range and preferably monochromatic
- Wavelength of light used should be at absorption maximum of the solution. This also gives the greatest sensitivity
- There must not be ionization, association, dissociation or solvation of the site concentration or time
- The solution is too concentrated, giving as intense colour. The law only holds upto a threshold maximum concentration for a given substance.

MEASUREMENT OF EXTINCTION

The earliest colorimeter on the human eye to match the colour of a solution with one series of coloured discs. The results obtained were too subjective and not particularly accurate, visual colorimeters are now of historical interest only.

The photoelectric cell is superior to the human eye and accessing the degree of absorption

of a colour and is more objective.

THE PHOTOELECTRIC COLORIMETER

The photoelectric colorimeter is used for measuring the colours by means of electric device.It is more preferred than visual colorimeter due to certain advantages as, great speed, eliminates errors from personal and physiological methods, small sample volume, unknown concentration from standard curves etc.

White light from a tungsten lamp passes through a slit, then a condense less to give a parallel beam which falls on the solution under investigation contained in an absorption cell. The cell is made of glass with the sides facing the beam is parallel to eachother in most cases, the cells are 1cm and will hold 3 ml of liquid comfortably.

Beyond the absorption of cell is the filter which is selected to allow maximum transmission of the colour absorbed. If a blue solution, the colour of the filter is therefore complementary to the colour of the solution under investigation in some instruments. The filter is located before the absorption ion. The filter gives narrow transmission bands and therefore approximately to monochromatic light.

The filter is choosen so that , Bear's law is obeyed. The light that falls on a photo cell which generates on electrical signal is increased in strength by the amplifier and the amplifier signal passes to a galvanometer or digit read out , which is caliberated with a logarithmic scale , so as to give absorbance reading. Directly photo cells used in such cheap colorimeter have a precision of about 0.6% . The blank solution is first put in the colorimeter and reading is adjusted to zero extinction that is followed by Test solution and extinction is read off.

A better method is to split the light beam, passes one part through sample and the other through the blank and determined from potentiometer reading which balance the circuit.

EXP NO:06
DATE:

ESTIMATION OF REDUCING SUGAR BY DNSA METHOD

AIM:

To estimate the amount of reducing sugar present in the given unknown sample.

REFERENCE:

1. An Introduction to Practical Biochemistry,3rd edition, By David.T.Plummer, Pg No: 180
2. HiPer Carbohydrates Estimation Teaching Kit (Quantitative).himedialabs.com/TD/HTBCOO3.pdf

REQUIREMENTS :

(i) Sodium hydroxide
(ii)DNSA reagent
(iii)Standard stock solution
(iv)Working standard solution
(v)Unknown sample
(vi)Boiling waterbath
(vii)Aluminium foil

WORKING FORMULA

(i) 2M sodium hydroxide

(ii) DNSA reagent-1gm of DNSA, 0.2g of phenol, 0.05g of sodium sulphate in 80ml distilled water. Add 5ml of NaOH and make upto 100ml.

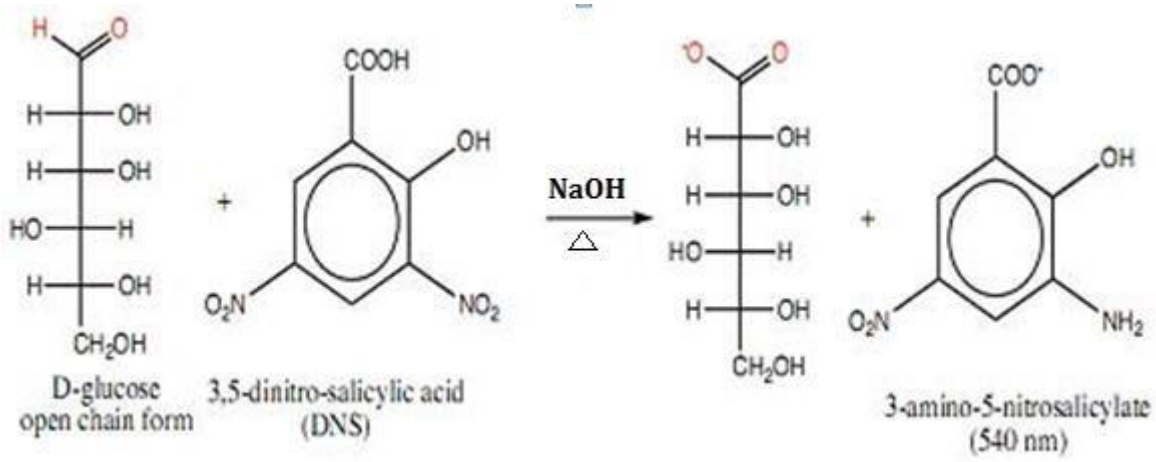
(iii) Standard stock solution-5g of glucose is dissolved in 100ml of water

(iv) Working standard solution-10ml of stock solution is diluted to 100ml with distilled water.

PRINCIPLE :

Carbohydrates are polyhydroxy aldehydes or ketones which forms enediol and are oxidized by 3,5-dinitrosalicylic acid . Several reagents are used in the assay of sugars by using their reducing properties. One such compound is 3,5-dinitrosalicylic acid (DNSA) which in alkaline solution is reduced to 3-amino-5-nitrosalicylic acid. DNSA method is for estimating the concentration of reducing sugars.Different sugars give different colours,so this method is not suitable for complex sugars.

The intensity of the orange colour changes on the concentration of reducing sugar involved in the reaction to find out the sugar content of the unknown sample. Compare the colour intensity developed by the unknown volume of sample with the colour gives graded quantities of sugar and thus calculating the concentration. The absorbance of the resultant solutions is read at 540 nm. The intensity of colour depends on the concentration of reducing sugar produced.



PROCEDURE:

Pipette out 0.2-1 ml of working standard solution whose concentration ranging from 1000-5000 micrograms to 1250 microgram in clean dry test tube. Mark as S_1 to S_5. Transfer 0.2 ml to 0.4 ml of unknown solution in tubes marked as T_1 and T_2. Made up the volume with distilled water upto 3 ml and take 1 ml of DNSA to all the tubes and cover the mouth of the tubes with aluminium foil. Keep those tubes in a boiling waterbath for 6 minutes. Cool under tap water, resultant colour is measured at 540 nm. Draw a standard graph with concentration of glucose on X-axis and optical density on Y-axis. Plot the values and the value of unknown sample was calculated.

SL NO:	PARTICULARS	S1	S2	S3	S4	S5	T1	T2	B
1.	Standard solution (ml)	0.2	0.4	0.6	0.8	1.0	-	-	-
2.	Concentration of the solution (mg//ml)	1	2	3	4	5	-	-	-
3.	Sample solution (ml)	-	-	-	-	-	0.2	0.4	-
4.	Distilled Water (ml)	3	3	3	3	3	3	3	3
5.	DNSA reagent (ml)	1	1	1	1	1	1	1	1
Cover the tubes ,keep in a boiling waterbath for 5 minutes ,cool under tap water and measure the optical density.									
6.	Optical Density (540 nm)								

FROM GRAPH :

A standard graph is plotted with concentration of glucose against optical density. From the values plotted the value of unknown sample was calculated as,

1) Sugar concentration in the Test sample T1=Concentration of unknown 'x' in µg/100ml

= x ×5µg/ml

2) Sugar concentration in the Test sample T2=Concentration of unknown 'x' in µg/100ml

= x ×5µg/ml

REPORT :

EXP NO:07
DATE:

ESTIMATION OF BLOOD GLUCOSE BY FOLIN-WU METHOD

AIM:

To estimate the amount of Glucose present in the blood sample by Folin-Wu method.

REFERENCE:

1. Laboratory Manual and Practical Biochemistry, By T.N. Pattabhiraman, 4th Edition, Pg No: 44-46.
2. Practical Biochemistry and Clinical pathology, S.R.Kale, R.R. Kale, Pg No: 40-44.
3. Practical Biochemistry and clinical pathology, M.S. Chaudri, S.B.Gokhale, Pg No: 66-68.
4. A Manual of Biochemistry, Dr.G.Deala Rao, 2nd Edition .Pg No:84
5. Biochemistry & Clinical Pathology- Theory & Practical, P.C.Dandiya, P.K.Sharma ,2nd Edition,Pg No:225.

REAGENTS REQUIRED:

1. .Alkaline Copper sulphate solution : Dissolve 40gm Anhydrous sodium carbonate, 7.5 gm tartaric acid in 400ml of distilled water.
2. Phosphomolybdic acid reagent : Dissolve 70gm molybdic acid, 10gm sodium tungtate, 400ml of 10% NaOH in 400ml of distilled water. Boil the solution for 30 minutes to remove ammonia. Cool the solution and add 125 ml of Phosphoric acid (specific gravity-1.75). Add distilled water to make 1 litre.
3. Standard glucose solution : Dissolve 100mg of glucose in 100ml distilled water (1mg/ml).
4. Working standard : Dilute 10ml of stock glucose solution to 100ml with distilled water (0.1 mg/ml)or (100µg/ml).Take 2 ml from this as working standard containing 0.2mg/ml of glucose.

PRINCIPLE:

This method is based on the reducing property of glucose. Alkaline copper reduction method is most widely used and it is very simple and rapid method. In this method, protein free filtrate (Folin-Wu method) by precipitating the proteins of blood by tungstic acid. So that 70ml of filterate corresponds to 1ml of blood sample. Then this protein free filterate containing glucose is heated with copper sulphate solution. Thus glucose reduces copper sulphate to form equivalent quantity of cuprous oxide.This cuprous oxide formed is reduced with phosphomolybdic acid to produce corresponding equivalent quantity of molybdenum blue.The molybdenum blue gives intense blue colour, the intensity of which is directly proportional to cuprous oxide which corresponds to the amount of glucose present in given sample of folin-wu filterate.

$C_6H_{12}O_6$ + Cu^{2+} → Cu^{+} + Oxidation products of glucose

Glucose cupic ion

$Cu^{+}Na_2PO_4$:12 MoO_3 + Cu^{2+} + 12 MoO

cupprous cupric molybdenum blue

CLINICAL SIGNIFICANCE:

- Glucose metabolism play a major role in carbohydrate, protein and fat metabolism
- Availability of glucose from various sources, its management and utilization is regulated by hormones of Islets of langerhans. Thus blood sugar level is maintained and normally expressed as fasting or post meal
- Normal value of blood sugar is 80-120 mg/dl.
- Fasting blood glucose level is 60-90 mg/ml. but according to this method it is about 80-120 mg/dl , which may be due to interfering substances like glutathione, other sulphydryl compounds, uric acid, glucuronic acid and ascorbic acid.

- Hyperglycemia ,is increased blood glucose level characterized by signs of diadetes mellitus or due to the lack of insulin or unfunctioning of β-cells to secrete insulin.
- In diabetes mellitus high values of fasting sugar values are obtained and vary from normal to 500 mg/100 ml and over according to severity of the condition.
- Hyperactivity of thyroid, pituitary, adrenal glands which include states of emotional stress etc increase blood sugar level about 150 mg%.
- Increased levels results in convulsions, infectious diseases, meningitis, tumours and haemorrhages.

PROCEDURE:

PREPARATION OF PROTEIN FREE FILTRATE

Pipette out 2.6 ml of isotonic sulphate solution to a centrifuge tube and add 0.4 ml blood, 0.5 ml of 10% sodium tungstate followed by 0.5 ml 2/3 N H_2SO_4. Mix well and allow to stand for 10 minutes followed by centrifugation. The colourless supernatant liquid is called protein free filtrate.

ESTIMATION OF BLOOD GLUCOSE

Mark three test tubes as Test 'T', Standard 'S' and Blank 'B'. Pipette 2ml of sample solution to test tube marked as T. Take 2 ml of standard solution (0.2 mg) to test tube S. Take 2 ml of distilled water to test tube B. Pipette out 2 ml of Alkaline copper reagent into T.S, and B. Keep the tubes in a boiling waterbath for 8 minutes. Cool and add to each test tubes 2 ml of Phosphomolybdic acid reagent. Mix well and make to 25 ml with distilled water with gradual mixing. Then by inversion, Read the optical density using a green filter at 540 nm.

OBSERVATION & CALCULATIONS :

SL NO:	PARTICULARS	STANDARD (S)	TEST (T)	BLANK (B)
1.	Volume of Standard solution (ml)	2	-	-
2.	Volume of distilled water (ml)	-	-	2
3.	Volume of Test solution (ml)	-	2	-
4.	Volume of Alk.copper reagent (ml)	2	2	2
		Keep the tubes in a boiling waterbath for 8 minutes,then cool.		
5.	Volume of Phosphomolybdic acid reagent (ml)	2	2	2
		Mix well and makeup to 25 ml mark with distilled water. Mix by Inversion and read the optical densities of each.		
6.	Optical density (O.D) at 540 nm.			

$$\text{Glucose level in 100 ml blood} = \frac{\text{O.D of T-O.D of B}}{\text{O.D of S-O.D of B}} \times 0.2 \times \frac{100}{0.2} \text{ mg}$$

RESULT:

The amount of Glucose present in the given sample is found to be ---------- mg/100ml

EXP NO:08
DATE:

ESTIMATION OF PROTEIN IN SERUM BY BIURET METHOD

AIM:

To estimate the total amount of protein present in the given sample by biuret method**.**

REFERENCE:

1. Practical Biochemistry and Clinical Pathology, S.R.Kale,R.R.Kale, Pg No: 25-26
2. A Manual of Practical Biochemistry, Dr. G. Devala Rao.Pg No:55
3. Laboratory Manual and practical Biochemistry , T.N.Pattabhiraman, 4th Edition, Pg No:47

REAGENTS:

i. Standard Sodium hydroxide (0.05µg/ml): Dissolve sodium hydroxide (0.5gm) in one litre of water.

ii. Standard protein solution (4mg/ml): Dissolve 5gm albumin in 250ml sodium hydroxide solution.

iii. Stock Biuret reagent: Dissolve sodium potassium tartarate (9g) in 500ml of 0.2N sodium hydroxide. To this add potassium iodide (5g). Dissolvenit completely and then add 3g copper sulphate. Dissolve it & dilute to one litre water.

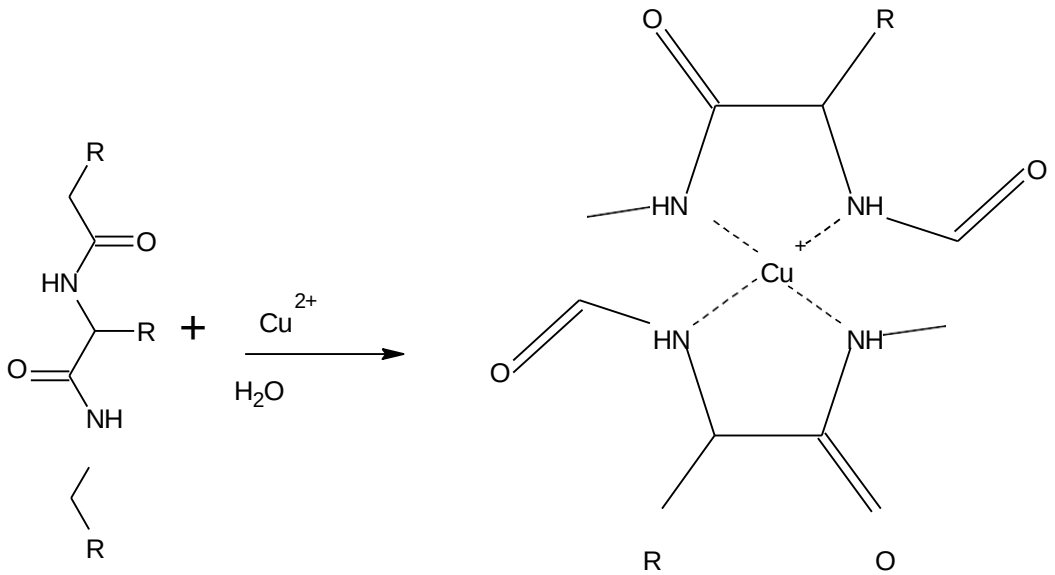
iv. Working standard : Dissolve 10ml of stoke solution in 100ml distilled water (0.1g).

REQUIREMENTS :

Colourimeter and Autoanalyser

PRINCIPLE:

The alkaline copper solution with bonds in protein, which is present in serum sample to form a violet colour complex. The violet colour complex is read at 660nm using photo electric colorimeter.The absorbance of the violet complex is proportional to the concentration of the protein present.



Peptide bond Cu^{2+}-Co-ordination complex

The intensity of colour was measured and was compared with the standard. Inorder to estimate the unknown protein concentration, a protein solution is used as standard to obtain the caliberation curve by plotting of optical density of unknown sample based on the grams, estimating the amount of proyein present in unknown sample.

Intesity of colour produced in biuret reaction is proportional to the number of peptide bonds, which in turn is a function of protein concentration.

PROCEDURE:

Pipette out 0.2ml of standard protein solution into a test tube. Concentration range from 20µg-100µg marked as S_1-S_5. The volume of the test tube made upto 4ml with distilled water. Take 0.2-ml and 0.4ml of unknown protein solutions in test tubes marked as T_1-T_2 and was made upto 4ml with distilled water. Add 6ml of Biuret reagent to each test tubes and are incubated at 37^0C for 10 minutes.

The colour intensity in each test tube is measured colourimetrically at 320nm against blank. Draw a standard graph with concentration of albumin on X-axis and optical density on Y- axis. Extrapolate the value of T_1 and T_2 based on graph. Calculate the amount of protein present in the given sample.

Advantages : Speed, simple, one step process and widely used method.

Disadvantages : Less sensitive with the minute quantities of protein even in mg samples. Reactions may be interfered by substances used often by glucose and other reducing substances.

NORMAL VALUE:

Total serum protein range from 6.5 to 8.0%. Albumin to globulin ratio 1:2:1. Sometimes it is 1:1.

CLINICAL SIGNIFICANCE:

- An increase in total protein may occur in dehydration. Albumin concentration increases very rarely.
- A decrease in total protein is always due to low albumin level. Low serum albumin may be due to nephrosis, malabsorbtion of protein into alimentary tract or decresed formation in liver or insufficient intake of proteins. Many times total serum protein remains same but albumin/ globulin ratio fails.

- In nephrosis heavy loss of albumin is seen, low serum is also found in severe liver disease due to impaired ability of the liver to produce albumin. Low albumin is one of the factors in causing edema in liver diseases.
- Increases in total protein in chronic infection, in advanced liver diseases and in multiple myeloma. Many times it reaches to 8 to 12%.
- Protein excretion can go upto 15gm per day under some conditions.

OBSERVATION & CALCULATIONS :

SL NO:	PARTICULARS	S	T	B
1.	Volume of Standard protein solution (2 mg/ml)	3	-	-
2.	Volume of Test protein solution (ml)	-	0.1	-
3.	Volume of Distilled water (ml)	-	2.9	3
4.	Volume of Working Biuret reagent (ml)	3	3	3
		Mix well and stand for 10 minutes and then read the optical densities.		
5.	Optical density /Absorbance (540 nm)			

1) Total protein in 100 ml of serum = $\frac{\text{Absorbance of Test} \times \text{Conc of Standard}}{\text{Absorbance of Standard} \times \text{Volume of Test}} \times \frac{100}{1000}$

$= \frac{\quad}{\quad} \times 6 \times \frac{100}{1000}$

$= \frac{\quad}{0.1}$ g/dl

2) Albumin in 100 ml 0f serum = $\frac{\text{Ab of test-Ab of Blank}}{\text{Ab of Std- Ab of Blank}} \times 100 \times 6 \times \frac{\text{g}}{0.1} \times \frac{\text{g}}{100}$

$= \frac{\quad}{\quad}$ g/dl

3) Globulin present =Total protein – Albumin present

4) Total protein in 100 ml of serum = $\frac{\text{Absorbance of Test} \times \text{Conc of Standard}}{\text{Absorbance of Standard} \times \text{Volume of Test}} \times \frac{100}{1000}$

$= \frac{\quad}{\quad} \times 6 \times 100$

$0.1 \quad 1000$

$= \quad$ ----------g/dl

5) Albumin in 100 ml 0f serum = $\frac{\text{Ab of test-Ab of Blank}}{\text{Ab of Std- Ab of Blank}} \times 100 \times \frac{6}{0.1} \times \frac{\text{g}}{100}$

=----------g/dl

6) Globulin present =Total protein – Albumin present

REPORT:

EXP NO:09

DATE:

ESTIMATION OF CREATININE IN BLOOD

AIM:

To estimate the creatinine in the given sample of blood.

REFERENCE:

1. Practical biochemistry and clinical pathology by Sharada R.Kale & Rajendra R. Kale 7^{th} edition
2. Laboratory Manual and practical Biochemistry , T.N.Pattabhiraman, 4^{th} Edition, Pg No:57

REAGENTS REQUIRED:

1. 1% Picric acid
2. 10% sodium hydroxide
3. Alkaline picraye solution-prepare just before use.
4. Stock creatinine solution: Dissolve 100mg of creatinine in 100ml of 0.1 N Hcl.
4. Working standard creatinine solution: Dilute 0.2ml of stock solution and 0.1 ml of conc.Hcl and make upto 100 ml with distilled water (0.2 mg/ml).

PRINCIPLE:

Creatinine in blood estimated by Folin Wu method by using photoelectric colorimeter. In this Folin Wu ie Protein free filtrate is used. The creatinine content in filtrate is treated with picric acid in alkaline medium to obtain red colour creatinine picrate. Optical density of red colour creatinine obtained is compared with that of standard solution. Similarly converted by

picric acid to creatinine picrate by using colorimetric principle concentration of creatinine in given blood sample can be calculated.

CLINICAL SIGNIFICANCE :

- Creatinine represents the waste products of creatine metabolism and it arises in the body from the spontaneous break down of creatine phosphate.
- It is a non-threshold substance and its excretion is considered as an index of endogenous protein metabolism. It is normally filtered by glomerulli.
- As its excretion is not related to any food protein, so its variations in the excretion indicate some of the metabolic disorders.
- Creatinine excreation increases during conditions like fevers, starvation, diabetes mellitus.
- Normal value of creatinine is 0.7-2.0mg/ml blood. Values below 90ml/minute indicates the diminished glomerular filteration rate.

PROCEDURE:

PREPARATION OF PROTEIN FREE FILTRATE:

Mark 3 cetrifuge tube as blank (B), test (T), standard (S). To the tube "T" 1ml of blood was added. Add 1ml of distilled water to the blank "B". Add 1ml of standard glucose solution to "S". Then add 7ml of distilled water, 1ml of sodium tungstate, 1ml of 2/3 N sulphuric acid to all the 3 test tubes. Mix well & allow to stand for 5min, centrifuge and collect supernatant protein free filtrate for estimation of creatinine.

ESTIMATION OF CREATININE:

Take 3 folin wu tube as blank (B), standard (S) & test (T). To each tube pipette out 5ml of corresponding protein free filtrate from the above centrifuge tube.To all the 3 tubes add 2ml of 1% picric acid and add 0.5ml of 10% sodium hydroxide, mix well and allow to stand for 15min. read the optical density at 530nm or using green filter in colorimeter.

OBSERVATION & CALCULATIONS :

SL NO:	PARTICULARS	S	T	B
1.	Volume of Working Standard solution (ml)	5	-	-
2.	Volume of Test solution (ml)	-	5	-
3.	Volume of Distilled water (ml)	-	-	5
4.	Volume of Alkaline picrate reagent (ml)	2.5	2.5	2.5
		Mix well and Incubate for 15 minutes at room temperature and then read the optical densities.		
5.	Optical density /Absorbance (540 nm)			

$$\text{Concentration of Test} = \frac{\text{Absorbance of Test} \times \text{Concentration of Standard}}{\text{Absorbance of Standard} \times \text{Effective Volume}} \times 100$$

$$= \frac{\text{Ab of Test}}{\text{Ab of Std}} \times \frac{0.01}{5} \times 100$$

=----------mg/100 ml

$$\text{Concentration of Standard} = \frac{0.2}{100} \times 5$$

$$= 0.01$$

REPORT:

The amount of creatinine present in the given sample of blood was found to be

----------mg/dl.

EXP NO:10
DATE:

ESTIMATION OF SERUM CHOLESTEROL BY ZAK'S METHOD

AIM:

To estimate the amount of Total Cholesterol present in the blood sample by libermannburchard's method.

REFERENCE:

1. Laboratory Manual and Practical Biochemistry by T.N.Pattabhiraman, Pg No: 51-52
2. Biochemistry & Clinical Pathology- Theory & Practical, P.C.Dandiya. P.K. Sharma, 2nd Edition , Pg No: 227-228.

REAGENTS REQUIRED:

1. $FeCl_3$- Acetic aicd reagent (stock solution): Dissolve 50 mg of $FeCl_3$-$6H_2O$ in 100ml of glacial acetic acid. Use fresh reagent for the estimation.
2. Standard cholesterol solution: Dissolve 100mg cholesterol in 100ml of glacial acetic acid in a standard flask and stored in a cool place.
3. Working standard of cholesterol solution: Dilute 1ml of the stock solution to 15ml with ferric-chloride-acetic acid reagent.
4. Test solution-Take 0.1 ml of serum and add 9.9 ml of ferric-chloride-acetic acid reagent.
5. Concentrated Sulphuric acid.

PRINCIPLE:

Libermann-Burchards first described a colour reaction for cholesterol using acetic anhydride and sulphuric acid. Acetic acid dissolves cholesterol which undergoes Libermann-Burchards reaction with acetic anhydride and sulphuric acid. The green colour that developed is proportional to cholesterol content of serum.

Zak's applied the principle for the development of a colorimetric method for assay of cholesterol. Precipitation of proteins in the procedure is essential since glyoxalic acid present as impurity in acetic acid, reacts with tryptophan in proteins to form a red colour under the assay conditions.Serum is deprotonized by the reaction with $FeCl_3$- Acetic aicd reagent . It is centrifuged and protein-free supernatant (containing $FeCl_3$) is treated with conc.H_2SO_4. The reddish purple colour is developed and the absorbance is measured at 540nm using yellow-green filter.

CLINICAL SIGNIFICANCE:

- Cholesterol level in the lipoprotein fraction, HDL is inversely related to the tendency of development of coronary heart disease. VLDL and LDL are precipitated by polyaniona like heparin or dextran sulphate.
- In healthy subjects, HDL cholesterol level in serum is above 45 mg/100ml in male and above 55mg/100ml in females.
- Normal total serum cholesterol level in young adults is in the range, 130-200 mg per 100ml.
- Cholesterol level increases with age.
- Increased level of cholesterol (hypercholesterolemia) include hypothyrodism, Uncontrolled diabetes, nephrotic syndrome, primary hyper lipoproteinemia (type II) and acute porphyria.
- Measurement of serum cholesterol level is clinically important, since hypercholesterolemia is a causative agent for artherosclerosis and coronary heart disease.

PROCEDURE:

Take 15ml centrifuge tubes. Add 0.1ml of serum and 9.9ml of $FeCl_3$- Acetic aicd reagent (working standard solution). Close the tubes properly and mix the contents. Let the tubes stand for 15 minutes at room temperature. Transfer 5ml of the clear supernatant into a dry 25ml conical flask marked as T(test). Into another flask marked S (standard), pipette 5ml of standard cholesterol solution (0.2 mg). As B (blank) take 5ml of ferric chloride-acetic acid reagent in a third flask. Add 3ml of concentrated H_2SO_4 into each flask. Mix by swirling. After 30 minutes read the optical densities (OD) using an yellow/green filter at 540-560nm range.

S.NO	CONDITION/DISORDER	SERUM CHOLESTEROL(mg %)
1.	Nephrosis	600-700
2.	Diabetes Mellitus	400- 500
3.	Coronary Thrombosis	300- 400
4.	Angina Pectoris	80- 100
5.	Hyper Thyrodism	Pernicious Anemia.

OBSERVATION & CALCULATIONS :

SL NO :	PARTICULARS	BLANK	TEST	STANDARD
1.	Volume of Standard solution (ml)	-	-	5
2.	Concentration of Standard solution (mg)	-	-	0.2
3.	Volume of Test solution (ml)	-	5	-
4.	Volume of Ferric chloride-acetic acid reagent (ml)	5	5	5
5.	Volume of Concentrated sulphuric acid (ml)	3	3	3
6.	Optical Density (540 nm-560 nm)			

Cholesterol present = Absorbance of Test × Concentration of Standard × 100

Absorbance of Standard × Effective Volume

=----------mg/100 ml

Concentration of Standard = 0.2 mg

Effective Volume = 5 ml

REPORT:

1. The Amount of Cholesterol in the given sample of serum was found to be------ mg/ml

EXP NO:11
DATE:

ESTIMATION OF HYDROLYTIC EFFECT OF AMYLASE ON STARCH

AIM:

To estimate the activity of **α**- amylase in a given sample of starch.

REFERENCE:

Laboratory manual in bio chemistry by J.Jayaraman.

REQUIREMENTS:

a) Phosphate buffer of pH6.8
b) Starch solution 1%
c) **α**- amylase solution
d) Sodium chloride 5%
e) Iodine solution
f) Distilled water
g) Water bath
h) Test tubes
i)

PRINCIPLE:

Amylase is an enzyme that hydrolases starch to maltose there are two types of amylase.

i. α- amylase- present in saliva and pancreatic juice.
ii. β- amylase – present in sprouted grain.

The process of digestion carbohydrates starts in the mouth by salivary **α**-amylase. **α**-amylase produce a random cleavage of glycosidic bond indicate the starch molecule to yield a mixture of maltose, maltobiose, glucose.In order to estimate the amylase, substrate starch or glycogen is allowed to hydrolyse with enzyme and product has been formed is measured. Salivary amylase is an α-amylase

that catalyses hydrolysis of **α-1,4**-glycosidic linkage of starch to amylase and amylopectin randomly. Hydrolysis of starch yields polysaccharides of gradually diminishing molecular size and ultimately achrodextrin and maltose. Various stages of hydrolysis of starch and changes of colour with iodine are as follows.

Starch ⟶ Amylodextrin ⟶ Erythrodextrin ⟶ Achrodextrin + Maltose

(Blue) (violet) (Red) (no colour)

Initially starch gives a blue colour with iodine, but on subsequent addition of iodine solution the blue colour changes to violet, red and then no colour there after.

PROCEDURE:

Take 5 ml of starch solution in a test tube. Add 2 ml of buffer and 1 ml of Sodium chloride solution. Incubate the mixture at 37ºC for 4-5 minutes. Take 0.5 ml of Iodine solution in 10-12 test tubes and place them in a test tube stand at room temperature. Now then add 2 ml of Salivary amylase to the tubes containg the reaction mixture, which was previously incubated. Mix it well.

Take a drop of this mixture and add it into the first test tube containing iodine. Repeat the same after every one minute, each time adding the mixture to the next test tube containing iodine placed in a row. Note the time taken to achive the achromic point with the iodine solution.

OBSERVATIONS & CALCULATIONS :

SL NO:	**PARTICULARS**	**T**									
1.	Volume of Starch solution (ml)	5									
2.	Volume of Buffer solution (ml)	2									
3.	Volume of NaCl (ml)	1									
	Incubate the mixture at 37ºC for 4-5 minutes										
4.	Volume of Salivary amylase (ml)	2									
	Test tubes	1	2	3	4	5	6	7	8	9	10
5.	Volume of Iodine solution (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6.	Achromatic point (minutes)										

REPORT:

The activity of the given sample on α-amylase was analysed and the achromatic point was found to be----------. .

EXP NO:12
DATE:

DETERMINATION OF EFFECT OF pH ON ACTION OF SALIVARY AMYLASE

AIM :

To determine the optimum pH on the activity of an enzyme**.**

REFERENCE:

1. Text book of Biochemistry by, DM Vasudevan, Sreekumari.S, Pg No: 46.

REQUIREMENTS :

- Enzyme solution
- Buffered substrate-1% starch solution
- Sodium chloride1%
- 2M Sodium hydroxide
- phosphate buffer of pH 7
- DNSA reagent
- Saturated maltose enzyme- 100 mg of maltose weighed and dissolved in 100ml of water.

PRINCIPLE:

Enzymes are biological catalyst and are active over only a limited range of pH. This is because; enzymes are made up of amino acids, many of which varies the ionisable side chain. So change in pH which affect the ionization of amino acid and which turns to affects the overall configuration of enzyme. Change in 3D structure of enzyme affect the binding of substrate molecule and hence the formation of enzyme substrate complex.

$$E + S \longleftrightarrow ES \longrightarrow E + P$$

The velocity of reaction is proportional to the concentration of ES, ie concentration of ES is critical in enzyme reaction. The pH of medium in which the reaction takes place affects the formation of ES complex. The pH affects the enzyme activity in 3 ways.

1. **It affects the ionization of side chain of enzymes:**

   The binding of substrate on enzyme take place at active centre and the binding is mainly electrostatic depends upon the substrate and active centre. Since the active centre is composed of amino acids charge on varied with pH. If follows the substrate binding to the enzyme will be affected.

2. **It affects the stability of enzymes**:

   An enzyme is fragile which cannot stand with enzyme pH. The extreme change in pH of medium, in which the enzymatic reaction carried out will adversely affect the confirmation and may cause the change in their structure and subsequent loss of activity.

3. **It affects the ionization substrate molecule:**

   To find out the pH at which enzymes show maximum velocity (optimum pH) Can be determined by placing the enzyme and substrate along with other critical factors at different pH. The step is followed by incubation of appropriate time interval and the resultant product is measuring in each enzyme solution by using alkaline DNSA. The pH at which the enzyme is mostly active considering as their optimum pH.

PROCEDURE:

The test tubes were obtained and cleaned thoroughly, dried and tubes were marked as **B, T_1, C_1, T_2, C_2, T_3, C_3, T_4, C4 and T_5&C_5.** Buffered substrate was prepared with a varying pH ie, pH- 2, pH- 4, pH-6, pH- 7, and pH- 9. 2.5 ml of each of buffered substrate was taken in test tubes (ie buffered pH-2 in **T_1& C_1** , pH- 4 in **T_2& C_2,** pH-6 in **T_3& C3,** pH- 7 in **T4&C4 and** pH-9 in **T_5&C_5**). 0.1ml of sodium chloride was added in each tube and the tubes were incubated at 37^0C for 10min. it was followed by addition of 0.5ml of saliva to **T_1, T_2, T_3, T4, and T_5.** T he tubes were incubated at 37^0C for 15min and then add 1ml sodium hydroxide of each tube and it was followed by the addition of 0.5ml of saliva to **C_1, C_2, C_3, ,C_4 and C_5.** Simultaneously,

5ml of distilled water taken as blank. Add 1ml of DNSA reagent to each tubes and kept the test tubes in boiling water bath for 5min. Resultant colour intensity was measured colorimetrically at 575nm. Optical difference was made and plotted the graph with pH on X-axis and absorbance on Y- axis. From the graph determine the pH at which the enzyme in mostly active.

OBSERVATIONS & CALCULATIONS :

SL NO:	PARTICULARS	B	T1	T2	T3	T4	T5
1.	Volume of Buffered substrate (ml)	-	1	1	1	1	1
2.	Volume of NaCl (1%) (ml)	-	1	1	1	1	1
		Preincubate for 5 minutes at 37^0C					
3.	Volume of Enzyme (ml)	-	0.1	0.1	0.1	0.1	0.1
4.	Volume of Distilled water (ml)	2	-	-	-	-	-
5.	Volume of 2N NaOH (ml)	1	1	1	1	1	1
6.	Volume of DNSA reagent (ml)	1	1	1	1	1	1
		Keep in a boiling waterbath for 10 minutes					
7..	Optical Density (540 nm)						

FROM GRAPH:

A standard graph is plotted with PH on X-axis and the measured Optical densities on Y-axis. Mark the optimum PH.

RESULT:

EXP NO:13
DATE:

DETERMINATION OF EFFECT OF TEMPERATURE ON ACTION OF SALIVARY AMYLASE ENZYME

AIM:

To determine the optimum temperature for the activity of an enzyme.

REFERENCE :

1. An Introduction to Practical Biochemistry, 3rd edition ,David T Plummer, Pg No:246.

REQUIREMENTS :

- Enzyme solution (10 mg diastase in 10 ml)
- Buffered substrate (10% starch solution)
- Sodium chloride1%
- 2M Sodium hydroxide
- phosphate buffer of pH 7
- DNSA reagent

PRINCIPLE:

Enzymes are sophisticated fragile molecule are greatly influenced by many parameter like pH, temperature, substrate concentration and enzyme concentration. Hence the temperature of medium influence up to a particular temperature beyond the point enzyme activity decrease, it may be due to heat denaturation of the enzyme and subsequent loss of tertiary structure. The temperature at which the enzyme attains Vmax termed as optimum temperature.

In order to find the optimum temperature the substrate and enzyme is incubating at different temperature and it is followed by the addition of DNSA. The resultant colour intensity was measured and the product in tubes which has been kept at different temperature shows the catalytic efficiency of enzyme.

PROCEDURE:

2ml of buffered substrate was taken in a test tubes marked as **B, T_1, C_1, T_2, C_2, T_3, C_3, T_4,C_4 and T_5&C_5.** 1ml of sodium chloride was added in each tube and the tubes were pre incubated for 5min at different temperature is **T_1, C_1** at 10^0C, T2**, C_2** at 25^0C, **T_3, C_3** at 37^0C, **T_4,C_4** at 45^0C, **T_5&C_5** at 100^0C. It was followed by the addition of 0.5ml of saliva to **T_1, T_2, T_3, T4, and T_5**. Then the tubes were incubated at this respective temperature for 15min. Add 1ml of sodium hydroxide to each tubes including blank which has been maintained at 37^0C. Then add 0.5ml of enzyme to **C_1, C_2, C_3, ,C_4 and C_5**. It was followed by the addition of 1ml of DNSA to each test tube and the tubes were maintained at boiled temperature for 6min. cool and added 5ml of distilled water to each tube. Measure the optical density of each tube against a blank. Optical difference was made and plotted the graph with temperature on X-axis and absorbance on Y- axis. From the graph determine the optimum temperature.

OBSERVATIONS & CALCULATIONS:

SL NO:	PARTICULARS	B	T1	C1	T2	C2	T3	C3	T4	C4	T5	C5
			10^0C		**25^0C**		**37^0C**		**45^0C**		**100^0C**	
1.	Volume of Buffered substrate (ml)	-	2.5	-	2.5	-	2.5	-	2.5	-	2.5	
2.	Volume of NaCl (1%) (ml)	-	-	1	-	1	-	1	-	1	-	1
		Preincubate for 5 minutes at 37^0C										
3.	Volume of saliva/ Enzyme (ml)	-	0.5	-	0.5	-	0.5	-	0.5	-	0.5	-
		Incubate for 5 minutes										
4.	Volume of NaOH (ml)	1	1	1	1	1	1	1	1	1	1	1
5.	Volume of Enzyme (ml)	-	-	0.5	-	0.5	-	0.5	-	0.5	-	0.5
6.	Volume of DNSA reagent (ml)	1	1	1	1	1	1	1	1	1	1	1
		Keep in boiling for 6 minutes										
7..	Volume of Distilled water (ml)	5	5	5	5	5	5	5	5	5	5	5
8.	Optical Density (540 nm)											

FROM GRAPH :

Plot a standard graph on Temperature on X-axis and Optical density values on Y-axis. Mark the optimum temperature in the graph

REPORT :

The Optimum temperature of an enzyme activity of salivary amylase was found to be ---

EXP NO:14
DATE:

DETERMINATION OF EFFECT OF SUBSTRATE CONCENTRATION ON ACTION OF SALIVARY AMYLASE ENZYME

AIM:

To determine the effect of substrate concentration on salivary amylase activity and to determine the K_M and V_{MAX} of the enzyme for a particular substrate, Where K_M is the substrate concentration at half V_{MAX}, V_{MAX} is the half maximum velocity of the enzymatic reaction.

REFERENCE :

REQUIREMENTS
:

- Starch solution of 0.5%, 1% & 2%
- Buffer of PH 6.8
- 5% Na Cl
- Iodine solution
- Distilled Water
- Amylase enzyme

PRINCIPLE:

The enzyme α-amylase can catalyse the hydrolysis of internal α-1,4-glycosidic bond present in starch with the production of reducing sugars. In the study of substrate concentration on enzyme kinetics, the enzyme is kept constant where as the concentration of starch is taken in increasing order. As the substrate concentration increases, the amount of products produced in every successive tube also increases.

This enzyme-substrate reaction can be determined by measuring the increase in reducing sugars using the DNSA reagent. In an alkaline condition, the pale-yellow coloured 3,5-dinitrosalicylicacid undergo reduction to yield orange coloured 3-amino-5-

nitrosalicylicacid. The absorbance of the resultant solution is read at 540 nm. The intensity of colour depends on the concentration of reducing sugars produced.

With a fixed concentration of enzyme an increase of substrate will result at first in a very rapid rise in velocity or reaction sate which give a hyperbolic curve. As the substrate concentration continues to increase, however the increase in rate of reaction beg instoslow down until, with a large substrate concentration, no further change in velocity is observed. Michealis and others reasoned correctly that an enzyme catalyzed reaction at varying substrate concentrations is diphasic i.e. at low substrate concentration the active sites on molecules (enzyme) are not saturated by substrateand the enzyme rate varies with substrate molecules concentration (phase1). As the number of substrate molecules increases, the sites are covered to a greater degree until at saturation no more rites are available, the enzyme is working at full capacity and now the rate is independent of substrate concentration. (Phase II).

CONCLUSION: As the substrate concentration increases, the activity increases. However after certainconcentration of substrate, the activity remains same because of saturation of Active site on Enzyme with substrate Molecules

PROCEDURE:

Prepare the salivary amylase enzyme. Take 3 test tubes and mark the as T1,T2 and T3. To each test tube add 2 ml of buffer solution and 1 ml of Sodium chloride solution. Now add to each 5 ml of Starch solution prepared as 0.5% to T1, 1% to T2 and 2% to T3. Incubate the testtubes at 37ºC for 3-4 minutes. Add 2 ml of salivary amylase to each mixture and test a drop of mixture from each tube with iodine solution separately. Repeat this test after every minute till achromic point is reached.

For the given enzyme value, increase in substrate concentration increases the time taken to reach the achromic point. Starch is hydrolyzed quickly at 0.5% concentration and it takes longer time for 1% concentration and still longer time for 2 % concentration. Results are obtained by plotting graphically.

OSERVATIONS & CALCULATIONS :

SL NO:	PARTICULARS	T1 (0.5%)	T2 (1%)	T3(2%)
1.	Volume of Buffer solution (ml)	2	2	2
2.	Volume of 5% NaCl solutions (ml)	1	1	1
3	Volume of starch solution (ml)	5	5	5
		Incunbate at 37ºC for 3-4 minutes		
4.	Volume of Salivary amylase (ml)	2	2	2
5.	Add the mixture (1-4) drop wise to iodine solution (ml)	Achromic point reached	Achromic point reached	Achromic point reached
6.	Achromic point reached (minutes)			

FROM GRAPH :

A standard plot of Starch concentration on X-axis and Achromic point reached (time taken) on Y-axis is drawn and determine the K_M & V_{MAX} Values.

REPORT:

The substrate concentration K_M at V_{MAX} is obtained as----------

SL NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**MOLISCH'S TEST** To 1ml of aqueous solution of sample in a test tube, add 2 drops of Molisch's reagent (Alcoholic solution of 2-naphthol) shaken well. Then 2 ml of con.H_2SO_4 is added carefully by the sides of test tube. **PRINCIPLE:** This is a general test for carbohydrate and is given by almost all sugars whether, free or double bond to such substance as protein (glycoprotein) and lipids (glycolipids). Only amino sugars fails to answer test. This is based on the fact polysaccharides and disaccharides are hydrolysis by conc.H_2SO_4 to form monosaccharides , then they undergo dehydration to form hydroxyl methyl furaldehyde, it condenses with naphthol to form a chromogen. The conjugated double bond system in the semiquinonoid formed is responsible for the colour.	A reddish violet coloured ring is formed at the junction of the two layers.	Indicate the presence of Carbohydrates.

2.	**SOLUBILITY TEST** Check the solubility of sample in water **PRINCIPLE:** Monosaccharides and disaccharides all soluble in water because they can form hydrogen binding with water. Polysaccharides are all insoluble because they donot form hydrogen binding with water.	Soluble Insoluble	Indicate the presence of Mono/Disaccharides. Indicates presence of polysaccharides.

3.	**IODINE TEST** Acidify the test solution with dil.Hcl then add one drop of Iodine solution, mix heat and then cool. **PRINCIPLE:** Starch, dextrin and glycogen can be differentiated by this test. The test depends upon the property of adsorption possessed by the large polysaccharide molecules which adsorb the smaller iodine molecules on their surface to form a complex or a well defined chemical nature. The starch-iodine complex is due to the formation of an inclusion complex between iodine and the amylase fraction of starch. Informing this complex, the lined amylase molecules into a spiral that is held by hydrogen bonding between hydroxyl glycerides of adjacent loops. The iodine molecule align themselves in the centre formed by the spiral. The arrangements allows the interaction of the electron orbital of many iodine atoms, which causes the light absorption that gives the	Blue colour on addition of iodine disappears on heating and reappears on cooling. Reddish purple colour on addition of iodine. Disappears on heating may or may not appear on cooling. Reddish brown colour on additionof iodine. Disappears on heating and disappears on cooling. No characteristic change.	Indicate presence of Starch Indicate presence of Dextrin Indicate presence of Glycogen. Indicate presence of Monosacharides/Disaccharides.

	complex its characteristic colour. The property of absorption decreases on heating and the complex dissociates and the colour disappears on cooling the colour appears except in case of dextrin. It is because the heating produce changes on the surface of the dextrin molecules as a result which they lose property of adsorption.		

4.	**FEHLING'S TEST** Take 2 ml of Fehlings solution A and Fehling solution B in a test tube. Add 1ml of test solution and boiled in a waterbath for 2 minutes. **PRINCIPLE**: This is general test for all reducing carbohydrates. This test is based on the fact that the carbohydrate are having a free aldehyde or ketone group and posseses reducing property just like the simple aldehyde and ketone. The reducing property of carbohydrate can be detected by several tests based on reducing of certain metal ions,eg: Cu, Fe, Sb, Ag, Hg etc by carbohydrates. Fehling A solution is $CuSO_4$ and Fehling B solution is KOH and sodium potassium tartarate in water. The $CuSO_4$ is hydrolysed to give cupric hydroxide ions. Cupric hydroxide is reduced to cuprous oxide on heating with a reducing sugar. $2Cu(OH)_2 \rightarrow Cu_2O + 2H_2O + (O)$ The colour of the solution changes from blue to red, as the carbohydrate also simultaneously oxidized to corresponding aldonic acid.eg: glucose	Brick red precipitate. No Brick red coloured precipitate.	Indicate presence of reducing sugar. Indicate presence of Non-reducing sugar.

	to gluconate. The reduction occur best in the alkaline medium which is provided by potassium hydroxide, sodium potassium tartarate. This keeps the cupric ions in the solution as cupric hydroxide which has a tendency to ppt . These test reaction is given by all monosaccharides, maltose & lactose.		

5.	**BENEDICT'S TEST** To 1 ml of aqueous solution of test sample add 2 ml of benedicts reagent and boiled in a test tube for 2 minutes. **PRINCIPLE:** The principle is similar to the fehling test. Aldoses and ketoses under alkaline condition tautomerises to enolol, are unstable and decomposes under alkaline conditions. The chain reaction continues to produce short chain aldehyde which are powerful reducing agent, that copper sulphate hydrolysis to form cupric hydroxide which is reduced to cuprous oxide on heating with reducing carbohydrates. Benedict's solution contains mild alkaline sodium carbonate. This produce alkaline medium and pp tot cupric hydroxide is checked by sodium citrate. All reducing sugars gives the tests positive, while sugar like sucrose doesnot give the test positive.	Brick red precipitate No Brick red precipitate.	Indicates presence of reducing sugar. Indicates absence of reducing sugar.

6.	**BARFOED'S TEST** To 2 ml of sugar solution add 2 ml of barfoeds reagent. Keep the tube in a boiling waterbath for 2 minutes. Cool under running water. Add phosphomolybdic acid dropwise and mix it till the solution is clear. **PRINCIPLE:** Barfoeds test is based on the reducing cavities of sugars. Aldose and ketose can reduce cupric ions in acidic conditions. Monosaccharides results fast, whereas reaction with reducing disaccharides is slow. Monosaccharides easilt to Cu^{2+} on treatment with phosphomolybdicacid which is blue in colour reducing disaccharides react weakly in this step.	A Deep blue colour is formed. Light blue colour is formed.	Indicate presence of Monosaccharides. Indicate presence of Disaccharides (maltose/lactose).

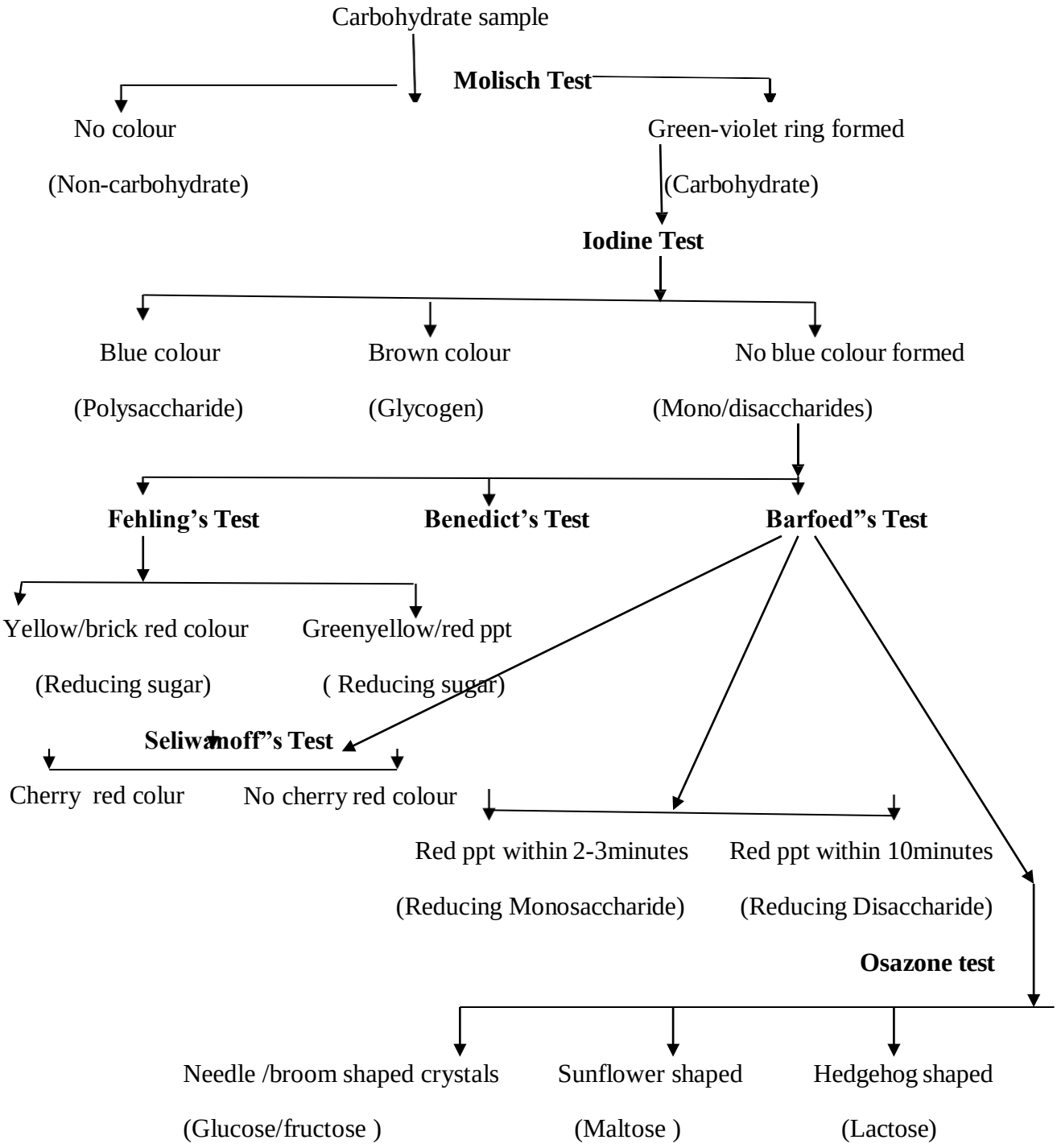
7.	**BIAL'S TEST** Add 2 ml of test solution in 5 ml of Bial's or citrol solution and heat for few minutes. **PRINCIPLE:** Pentoses are dependent and dehydrate by conc.HCl and resultant produce furfural contents with orcinol in presence of Fe^{3+} to form a green colour produces. Bial's reagent consists of 1.5 gm of resorcinol in 500 ml of conc.Hcl, add 20-30 drops of $FeCl_3$.	Green colour is formed (within 10 minutes)	Indicate presence of pentose.

8.	**BENEDICT'S TEST AFTER HYDROLYSIS** To 1 ml of solution add 5 drops of conc.Hcl, mix and boil for 2 minutes, cool and neutralize the solution by adding conc.Hcl. Mix solution by NaOH solution and check the PH using litmus paper. **PRINCIPLE:** Sucrose is readily hydrolysed to fructose and glucose by hot dilute mineral acids. There the monosaccharides answer the test, starch and dextrin are also hydrolysed to extend by acid. More reducing sugar gives a distinct positive test.	Red precipitate is formed Orange precipitate is formed.	Indicate presence of polysaccharides. Indicate presence of starch and dextrin.

9.	**SELIWANOFF'S TEST** To 1 ml of sample add 5 ml of seliwanoffs reagent and boil for 30 seconds. **PRINCIPLE:** Seliwanoff's reagent contains resorcinol in 6N Hcl. The principle of the test is based on the formation of hydroxymethyl furaldehyde and it condenses with resorcinol to form a chromogen. Fructose undergo dehydration under the acidic condition used, much more rapidly than glucose to yield the furaldehyde derivatives. Sucrose in hot Hcl solution is easily hydrolyzed to glucose and fructose and the later answer the test. In place of resorcinol aromatic alcohol can be used. The test is useful in differentiating aldohexose and ketohexose.	Appearance of Cherry red colour.	Indicates the presence of ketose (may be fructose/ sucrose)

10.	**OSAZONE TEST** Take a small quantity (200-100 mg) of phenyl hydrazine in a test tube, add a few sample, sodium aetate buffer and add 5 drops of glacial acetic acid and warm gently to dissolve the solid heat in the test tube in a boiling waterbath. After 20 minutes cool the solution and observe under a microscope. **PRINCIPLE:** All reducing sugars, osazones with excess of phenylhydrazine when kept in boiling temperature, hydrazine can highly soluble, but osazones of each sugars are insoluble and form characteristic crystals. The difference in the glucose, fructose, maltose depends on the first and second carbon atom and when the osazone is formed, the difference is marked. Hence these sugars will produce the same needle shaped crystals arranged like shears of com. The osazone of galactose has characteristic shape.	Small needle shaped crystals are formed. Hedge hog shaped crystals are observed. Sunflower shaped crystals are noted.	Indicate presence of Glucose/fructose. Indicate presence of Lactose. Indicate presence of Maltose.

The Scheme below explains the different Identification tests to be performed:



QUALITATIVE ANALYSIS OF CARBOHYDRATES-SAMPLE NO: 1

Sl No:	EXPERIMENT	OBSERVATION	INFERENCE
1.	**MOLISCH'S TEST** To 1ml of aqueous solution of sample in a test tube, add 2 drops of Molisch's reagent (Alcoholic solution of 2- naphthol) shaken well. Then 2 ml of con.H_2SO_4 is added carefully by the sides of test tube.		
2.	**IODINE TEST** Acidify the test solution with dil.Hcl then add one drop of Iodine solution, mix heat and then cool.		
3.	**FEHLING'S TEST** Take 2 ml of Fehlings solution A and Fehling solution B in a test tube. Add 1ml of test solution and boiled in a waterbath for 2 minutes.		
4.	**BENEDICT'S TEST** To 1 ml of aqueous solution of test sample add 2 ml of benedicts reagent and boiled in a test tube for 2 minutes.		

5.	**BARFOED'S TEST** To 2 ml of sugar solution add 2 ml of barfoeds reagent. Keep the tube in a boiling waterbath for 2 minutes. Cool under running water. Add phosphomolybdic acid dropwise and mix it till the solution is clear.		
6.	**SELIWANOFF'S TEST** To 1 ml of sample add 5 ml of seliwanoffs reagent and boil for 30 seconds.		
7.	**OSAZONE TEST** Take a small quantity (200-100 mg) of phenyl hydrazine in a test tube, add a few sample, sodium aetate buffer and add 5 drops of glacial acetic acid and warm gently to dissolve the solid heat in the test tube in a boiling waterbath. After 20 minutes cool the solution and observe under a microscope.		

SL NO	EXPERIMENT	OBSERVATION	INFERENCE
I. a.	Appearance of solution	Turbid	Albumin, globulin, casein
		Clear solution	Gelatin, peptone
b.	Colour of the solution	Opalescent or milky	Albumin, casein
		Faint yellow	Peptone
		Faint Brown	Gelatin
c.	Smell	Egg smell	Albumin
		Milk like smell	Casein
		Meat like smell	Peptone, gelatin
d.	Litmus paper test	Neutral	Albumin, gelatin
		Acidic	Peptone
		Alkaline	Casein & metaprotein

II.	COLOUR REACTION		
a.	**BIURET TEST** To 2 ml of sample solution was mixed with 2 ml of 10/NaOH & added 2-3 drops of 1% $CuSO_4$ solution and mix. PRINCIPLE: This is specific for peptide bond. Since all protein containpeptide linkage respond to this test by producing a violet colour between cupric hydroxide & peptide bond. The reaction is so named because the compound biuret (H_2N-NH-CO-NH_2) also answer the test. The colour of the reaction varies from pink to purple to violet depending upon the number of peptide groups present in the protein.	Violet or pink colour Rosy pink colour	Presence of protein Presence of peptone.

b.	**NINHYDRIN TEST:** To 1 ml of sample solution add 10 drops of Ninhydrin reagent (0.25% in acetone) and boil for 2 minutes PRINCIPLE: The Ninhydrin react with the 2 amino group of an aminoacid. Ninhydrin is reduced to hydrindatin while aminoacid is converted into an aldehyde having one carbon atom less than the aminoacid, CO_2, NH_3. Reduced ninhydrin (Hydrindatin) and NH_3 reacts with another molecule of ninhydrin to form a purple blue complex. Both biuret and ninhydrin test are general test for protein and is given by albumin, casein and even peptone.	Formation of violet colour	Presence of protein.

c.	**XANTHOPROTEIC TEST** To 2ml of sample solution add 1ml of Conc. HNO_3, heat the solution for 1min. cool under tap water.divide the contents in to 2 parts, to the second part add 40% NaoH. PRINCIPLE: This is a test for amino acid containing aromatic benzene ring like tyrosine, tryptophan and phenyl alanine. This amino acid undergoes nitration on treatment with strong nitric acid at elevated temperature. The nitrated derivative thus formed impact a yellow color to the solution. On addition of alkali, the nitro phenyl group ionizes impacting an orange color.	Yellow colour in alkaline medium. Orange colour in alkaline medium.	Presence of tyrosine,tryptophan and phenyl alanine. Presence of tyrosine, tryptophan & phenyl alanine.

d.	**MILLON'S TEST:** To 1ml of sample solution add 1ml of mercuric sulphate solution (10% sol in 10% H_2SO_4) boil gently to 30 sec. add 2 drops of 1% sodium nitrate. PRINCIPLE This test is answered by compound containing hydroxyphenly group which give red color due to the formation of mercury phenyled ion with the nitrated phenol radical tyrosine.	Red color develops	Indicates the presence of protein containing amino acid tyrosine.
e.	**ALDEHYDE TEST (HOPKIN – COLE TEST)** To 1 ml of sample solution add 2 drops of 0.2% formalin, mix and 2 drops of mercuric sulphate solution carefully and 1 ml of conc.H_2SO_4 through the sides of the tube with slanting position. PRINCIPLE: This is a test for Indole nucleus. Aldehyde react with the oxidized product of the indole nucleus of tryptophan in the presence of conc.H_2SO_4 to give violet coloured complex. Here H_2SO_4 with mercuric sulphate act as an oxidizing agent.	Violet color develops	Indicate presence of protein containing aminoacid Tryptophan.

f.	**ARGININE TEST (SAKAGUCHI TEST)** To 2 ml of sample solution add 1 ml of 10% NaOH, 5 drops of 1% of 2-naphthol and 5 drops of freshly prepared hypobromide solution. PRINCIPLE: Thisis a test for guanidine group. Free arginine residue in protein reacts with α-naphthol and alkaline sodium hypobromite to give red condensation product usually red-orange. Albumin answer positively to this test.	Intense Red colour	Indicates the presence of protein containing aminoacid,arginine.

III.	**SULPHUR TEST** To 1 ml of protein solution add an equal volume of 40% NaOH and boil at least for 3 minutes. Cool and add 2-3 drops of lead acetate. PRINCIPLE: When aminoacid like cysteine and cystine are boiled with strong alkali, the organic sulphur present in them is converted into sulphide addition of leadacetate to the solution cause the precipitation of insoluble leadsulphide which is black in colour. $R\text{-}SH + 2NaOH \rightarrow R\text{-}OH + Na_2S + H_2O$ $Na_2S + (CH_3COO)_2Pb \rightarrow PbS +$ $2CH_3COONa$(Black ppt)	Black pptate is formed.	Indicate the presence of protein containing aminoacid cysteine & cystine.

IV.	**PAULY'S TEST:** To 0.5 ml of sulphanilic acid solution add equal volume of 0.5% sodium nitrate solution mix well. After standing for a minute, mix well & add 2 ml of 10% sodium carbonate.	Red colour develops.	Indicates the presence of protein containing aminoacid histidine & hydroxyphenyl group of tyrosine.
V.	**MOLISCH'S TEST:** To 1 ml of sample solution add 2-3 drops of ethanolic solution of α-naphthol. Mix well and add 1-2 ml of conc.H_2SO_4 along the sides of the test tube. PRINCIPLE: This is a general test for carbohydrate and also answered by glycoproteins. Only amino sugar fail to answer this test. When carbohydrates are exposed to conc.H_2SO_4 in the presence of heat they undergo dehydration to form furfural derivatives. Hexoses form hydroxyl methyl furfural. It condenses with α-naphthol to form a chromogen. The conjugated double bond system in the semiquinonoid form is responsible for the colour.	A violet colored ring is formed at the junction of 2 liquids.	Indicate presence of glycoprotein.

VI.	**EFFECT OF HEATING:** Sample solution to be analysed is subjected to heating. PRINCIPLE: When protein is heated its physical, chemical and biological properties are changed due to breaking up of certain bond and resultant changes in the conformation. This process is known as denaturation. However coagulable proteins when heated at this isoelectric PH a series of changes occurs, involving • Dissociation of protein (disruptionof quaternary structure) • Uncoiling of polypeptide chain (disruption of tertiary & secondary structure) • Matting together of the uncoiled polypeptide chains (coagulation) • Chlorophenol red is used to adjust the PH of the solution to the isoelectric PH. Chlorophenol red gives a pink colour at this PH, a purple red about it and a yellow colour below it.	Coagulation occurs No coagulation	Indicates presence of albumin or globulin. Indicates presence of casein, peptone or gelatin.

VII.	**PRECIPITATION REACTIONS OF PROTEINS**		
A.	PRECIPITATION BY HEAVY METALS:		
a.	2 ml of alkaline sample is mixed with 5% of $CuSO_4$ solution.	The sample solution is made alkaline by using bluish white ppt soluble in excess but reappears on adding still more $CuSO_4$ solution.	Presence of protein.
b.	To 2 ml of above solution add 2-3 drops of 2% lead acetate solution.	White ppt is formed.	Presence of protein.
c.	To 2 ml of sample add 2 drops of 5% mercuric nitrate solution.	Brown ppt formed.	Presence of protein.
	PRINCIPLE: When solution of lead acetate, mercuric nitrate and other heavy metallic substance added to protein solution, interact with negatively charged group of the protein causing pptation as metal proteinate.		

B a.	**PRECIPITATION BY SALT:** **HALF SATURATION TEST WITH AMMONIUM SULPHATE** To 2-3ml of sample solution add an equal volume of saturated ammonium sulphate, mix well and allow to stand for 5 minutes. Filter & perform biuret test with the filterate using an equal volume of 40% NaOH and 2 drops of 1% $CuSO_4$ solution.	• Formation of violet colour • No violet or purple colour • No rosy pink colour	Presence of albumin. Presence of globulin, casein or gelatin Presence of peptone.
b.	**FULL SATURATION TEST WITH AMMONIUM SULPHATE:** To 3 ml of sample solution add solid Ammonium sulphate till the solution is saturated & allow standing for 5 minutes. Filter & perform biuret test with the filterate using an equal volume of 40% NaOH and 2 drops of 1% $CuSO_4$ solution.	Formation of violet colour.	Presence of Albumin.

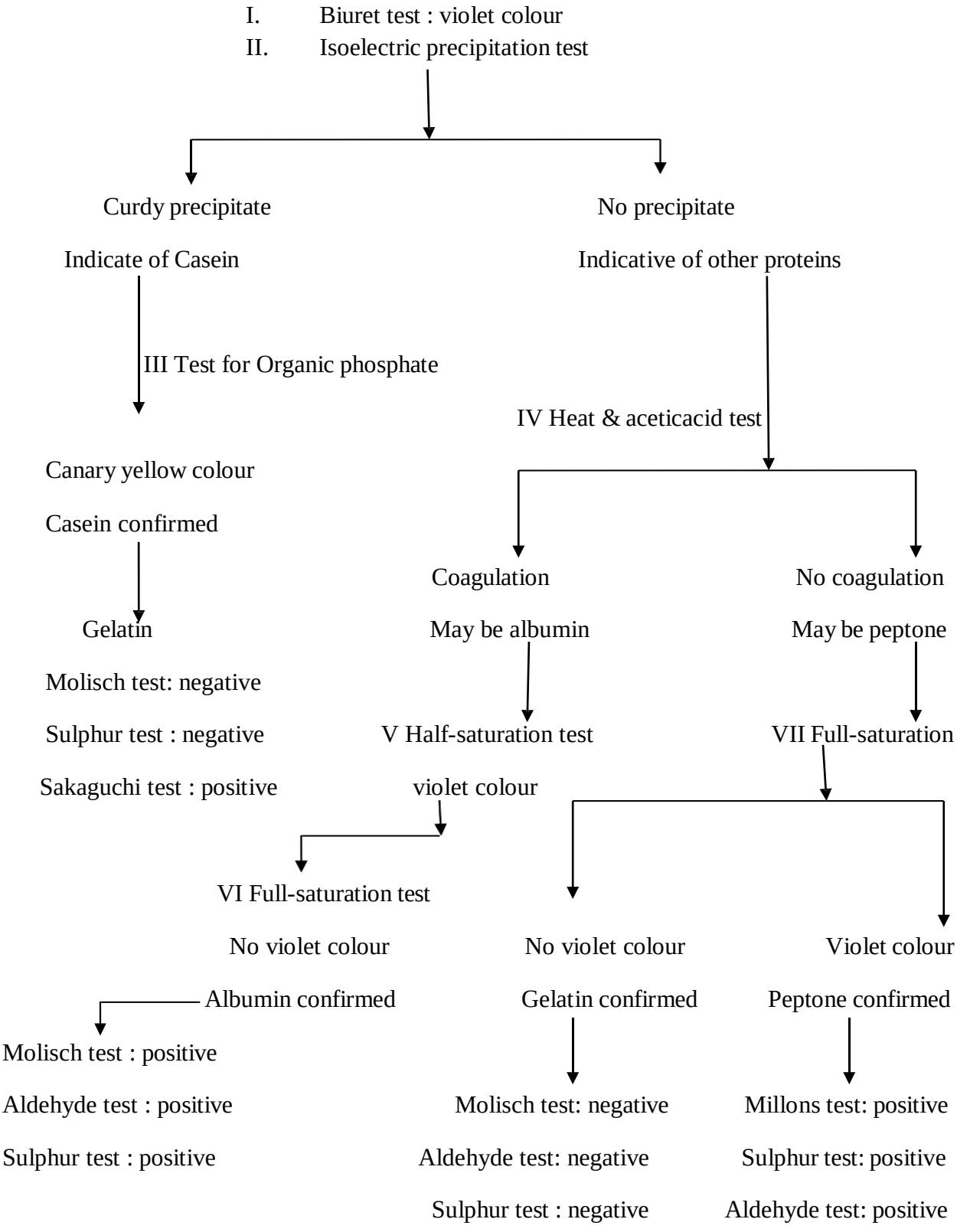
c.	**HEAT AND ACETIC ACID TEST:** To 10 ml of sample solution in a test tube and hold it above a flame in a slanting position and boil for few minutes. Boil upper 5 ml of solution and lower half serves as control. PRINCIPLE: Protein in solution is denatured when subjected to heating and the denatured protein are generally less soluble. Albumin starts precipitating when it is heat denatured. Addition of aceticacid fastens this process.	Cloudy white ppt in the heated portion and coagulation takes place when a few drops of 1% aceticacid is added.	Indicates the presence of albumin.

VIII	**OTHER TEST FOR PROTEINS:**		
1.	**NEUMAN'S TEST :**		
a.	To 5 ml of sample add 3 drops of CPR indicator. Then the solution turns pink. Then add 1% aceticacid drop wise till colour change to yellow.	Precipitate appears	Presence of Casein.
b.	To above ppt add 3 drops of conc.H_2SO_4 and 10-12 drops of conc.HNO_3. Heat until colour produces. PRINCIPLE: When an organic salts like ammonium sulphate is added to a solution of protein the effective concentration of water available for the protein is decreased and the protein is ppted.Colloidal protein are kept in solution by 2 factors. 1. Electric changes: changes on protein 2. Shell of hydration: film of water surrounding the protein If both these factors are removed the particle and are ppt. this can be done by adding a neutral salt (NH_2SO_4) which neutralizes electric changes and remove hydration shell. The	Shining yellow or canary yellow colour is produced.	Presence of Casein.

	amount of NH_3SO_4 depends upon the surface area of the particles. • Albumin: it is a small molecule; hence surface area is large so ppted by full saturation only. • Casein & gelatin: large molecule has small surface area. Soil is ppted by both half & full saturation. Peptone: very small, Therefore no saturation occurs		

2.	**ISOELECTRIC PRECIPITATION OF CASEIN:** To 3 ml of sample solution add 2 drops of bromocresol and add 1% aceticacid dropwise until a green colour solution is obtained. PRINCIPLE: Protein have minimum solubility at their isoelectric point as the protein molecules became electrically neutral at this PH. Most protein can be ppted by heating at this PH or by adding sufficiently amount of acid or alkali. Bromocresol green is used to indicate PH in the test. At a PH of 5 or above.	White curdy ppt is formed.	Presence of Casein.
3.	**HELLER'S TEST:** To 2 ml of conc.HNO_3 add 2 ml of protein solution from the sides of the test tube without disturbing the contents. PRINCIPLE: Conc.mineral acids, such as conc.HNO_3 convert the native protein molecule into soluble meta-protein. Note: This test is not given by derived proteins.	A white ring is formed at the junction of 2 liquids.	Indicate presence of protein, albumin.

he General Scheme for the Identification of an Unknown Protein sample :



RINE ANALYSIS (QUALITATIVE)

- Urine is the chief excretory fluid eliminated through the kidney. Most of excretory products are eliminated through urine. Urine shows the presence of large number of organic and inorganic constituents. The composition of urine varies largely and mainly depends on nature of diet the individual take place.

- Apart from there variations any sample or normal urine contains organic and inorganic constituents like chloride, sulphate, phosphate, urea, uric acid and creatine. They are defined as low threshold substances as get filtered very easily during glomerular filtration.

- The abnormal or pathological urine is that sample contains essential of body like glucose, ketones. bile salts and protein etc. these are defined as high threshold substances are not filtered easily during glomerular filtration.

Collection of sample:

For quantitative analysis it is necessary to collect 24 hours. Sample analysis is then carried out on measured amount. The urine is collected in a large bottle to which 10ml toluene is added as a preservative.

Volume:

Quantity of urine formed in 24hours in an adult normal individual varies from 6000ml to 2500ml. Normally it depends upon water intake, diet, environmental temperature,mental etc.

Colour:

Normal colour of urine is pale yellow or amber is mainly depends upon presence of urochrome.

Reaction:

Normally freshly voided urine is usually clear and acid in reaction with pH value as low as 4.5 and as high as 8.2.

Specific gravity:

It varies from 1.01 to 1.05

Turbidity:

Usually fresh urine is transparent , pathological sample may be turbid due to presence of mucoid, epithelial, pus cells etc. Normally it is faintly aromatic and is neutral with smelling substance "urinod".

NORMAL CONSTITUENTS OF URINE:

A) ORGANIC CONSTITUENTS OF URINE:

1. **Urea:** It constitutes about half of total urinary solid. It is the main end product of protein metabolism. In human being it represents about 80-90% of total urinary nitrogen. About 25-30gm of urea are excreted per24 hours. In liver diseases urea level is decreased.

2. **Uric acid:** It is end product of purine metabolism. About 0.7gm uric acid is excreted through urine per 24hours. It is derived from breakdown of cellular nucleoprotein. Excretion of uric acid is increased in leukemia severe disease and in varies states of gout.

3. **Creatinine:** Creatinine is the product of breakdown of creatine. The amount of creatinine excreted through urine by an adult individual is 1.2 to 1.7gm in 24 hours.

B) INORGANIC CONSTITUENTS OF URINE:

1. **Chlorides:** It is the main solid constituents of urine. Generally 6-9gm/24hours as chloride excreted through urine. Excretion of chloride is mainly dependent on diet. In fever , nephrits, diarrhea, chloride excretion is decreased.
2. **Phosphate:** The amount of phosphate excretedthrough urine depends on diet. It is normally 0.8 to 1.3 gm of phosphate per day. In certain bone diseases excretion of phosphate is increased while in hypoparathyroidin it is decreased.
3. **Sulphate:** It is derived from metabolic degradation of sulphur containing amino acids. Excretion depends on breakdown of tissue protein. It is excreted about 0.7 to 1gm per day.

C) ABNORMAL CONSTITUENTS OF URINE:

1. Protein: The presence of protein in urine is known as " proteinurea". The protein commonly found in urine are albumin and globulin. Very rarely fibrinogen may also be present. They are derived from plasma. Generally albumin and globulin are present together but the amount of albumin, is much than that of globulin. The proteinurea may be divided into 2 types.

a. **Functional proteinurea:** This is not associated with any disease. Usually it is temporary and harmless. It may occur after severe exercise, after a cold bath and in pregnancy.

b. **Organic protein urea:** This is due to some definite organic disease. It may be divided into pre-srenal, renal and post renal depending upon the site of disease.

2. **Carbohydrates:** Glucose is present even in normal urine but its concentration is too low to be detected by the oridinary tests. The presence of detectable accounts of carbohydrates in urine is known as glycosurea. The carbohydrate which is most commonly found in urine in glucose. The common cause of glycosurea are :

i. Diabetic mellitus

ii. Emotional or stress glycosurea. This is due to hyper secretion or epinephrine under emotional stress.

3. **Ketone bodies:** The ketone bodies include **acetoacetate, acetone, β**-hydroxy butyrate. When the rate of formation of ketone bodies increase the extrahepatic oxidative machinery is saturated and blood level of these substance increases(ketoanemia). They are then xtracted in urine in detectable amount(ketourea) . These excessive production are known as ketosis.

4. **Bile pigments:** Bile salts are formed in the liver and are excreted in liver bile. They regurginate into systemic circulation in hepatic and pest hepatic jaundice due to intra or extra hepatic bilary obstruction.

5. **Blood**: The presence of intact RBC in urine is known as " haemoglobin in urine known as " haemoglobinuria". Haematuria occurs in cancer, stone in urinary tract, bleeding disorders such as haemophilia, purpura and leukemia.. haemoglobinuria occurs in haemalytic anemia, typhoid fever and blood transfusion.

PHYSICAL PROPERTIES

SL NO	TEST	OBSERVATION	INFERENCE
I.	**PHYSICAL EXAMINATION**		
a.	VOLUME	a)1000ml-1500ml/day b)Morethan1500ml/day c)Lessthan 1000ml/day d) No urine	Normal i)Polyuria may be due to more water intake,less perspiration, high protein dietwhich may be due to (a) Hard physical work (b) Fever (c) Dehydration (d) Vomoting ,diarrheoa (e) Acute nephritis
b.	COLOUR	a)Pale yellow b)Marked yellow c)Light yellow d)Yellowish green e)Reddish f)Dirty bluish g)Milky h)Brown colour i)Cloudy	Presence of Urochrome pigment. Decreased urine output. Seen after heavy meals. Jaundice. Haematuria. Cholera & typhus. Pyuria & chyluria. Abnormal. Ppt of urate phosphates etc.

c.	ODOUR	a) Aromatic b) Unpleasant	Due to various drugs. Due to ketonsis.
d.	SPECIFIC GRAVITY	a) 1.012-1.024 b) Less than 1.0212 c) More than 1.024	Normal Due to dilution or diabetes insipidus. Due to perspiration or diabetes mellitus.
e.	PH	a) 6-7.5 b) Acidic (below 6) c) Alkaline (above 8)	Normal After high meat diet and in acidosis. Abnormally seen in alkalosis.

II	TEST FOR INORGANIC CONSTITUENTS		
1.	**TEST FOR BICARBONATES:** To 3 ml urine + dil Hcl or dil.H_2SO_4	Effervescence of CO_2 gas.	Presence of Bicarbonate.
2.	**TEST FOR CHLORIDES:** To 5 ml of urine + 1 ml of conc.HNO_3 + 1 ml of $AgNO_3$ solution.	White curdy ppt of AgCl soluble in NH_4OH solution.	Presence of chloride.
3.	**TEST FOR PHOSPHATES:** To 3 ml of urine + 3 ml conc.HNO_3 + 3 ml of ammonium molybdate solution and heat to boil.	Canary yellow ppt.	Presence of phosphate.
4.	**TEST FOR SULPHATES:** To 5 ml urine + 1 ml of conc.Hcl+ 2ml $BaCl_2$ solution.	An opaque milkiness or a thick white ppt of $BaSO_4$ insoluble in conc.Hcl.	Presence of sulphate.
5.	**TEST FOR AMMONIA:** a) To 5 ml urine + 2 ml 40% NaOH + boil and hold a litmus paper in the vapour. b) Dip a glass rod in	Red litmus paper turns blue. The colour becomes pink.	Presence of ammonia. Presence of ammonia. .

	a) phenolphthalein indicator and hold on fumes.	White ppt of calcium oxalate.	Presence of calcium
6	**TEST FOR CALCIUM:** To 5 ml urine + few drops of NaOH + 1% acetic acid + 2-3 ml of ammonium oxalate solution.		

III	TEST FOR ORGANIC CONSTITUENTS		
1.	**TEST FOR UREA:**		
	a) 3 ml of urine + few drops of alkaline sodium hypobromate solution.	Effervescence of nitrogen gas.	Presence of Urea.
	b) 5 ml of urine + 4 drops of phenolphthalein + pinchfull of ureas powder (jack bean/soybean meal) and mix. Allow to stand for 5 minutes.	Solution becomes pink, the pink colour decolourise on adding 10% aceticacid.	Presence of Urea.
2.	**TEST FOR URIC ACID:**		
	a) Moisten a strip of filter paper with $AgNO_3$ solution add it a drop o urine.	Black or yellow brown stain formed.	Presence of Uric acid.
	b) 5 ml of urine + 5 drops of benedicts uricacid reagent + 3 gms of anhydrous Na_2CO_3 and mix by shaking.	Deep blue colour develops.	Presence of Uric acid.
3.	**TEST FOR CREATININE:**		
	a) Weyl's test- 5 ml of urine +		

	5 drops of sodium nitroprusside + 2 ml of 10% NaOH. Jaffes test-5 ml of urine + 1 ml of saturated solution of picric acid + 3 gm of anhydrous Na_2CO_3 mix well by shaking	Ruby red colour is formed and changes to yellow. Deep orange colour is formed.	Presence of Creatinine. Presence of Creatinine

V.	**TEST FOR ABNORMAL CONSTITUENTS**		
1.	**TEST FOR PROTEINS:** a) Salphosalicylic acid test: 3 ml urine + sulphosalicylic acid drop by drop b) Heller's nitric acid ring test : 3 ml of conc.HNO_3 + add from side of test tube dropwise urine sample. c) Heat-coagulation test : 5 ml of urine + 2 drops of chlorophenol red, adjust the pH faint by adding 1% aceticacid or 2% Na_2CO_3, boil for 2 minutes.	White precipitate White ring at the junction of two fluids. Turbidity or precipitates	Albumin is present Albumin is present Albumin is confirmed.
2.	**TEST FOR SUGARS:** a) Benedict's test: 5 ml of urine + 5 ml of benedicts reagent, boil for 2 minutes and cool.	Green precipitate Yellow precipitate Red precipitate	1% Glucose present 2% Glucose present 2% or more than Glucose present.

	Fehlings test: 2 ml fehling A + 2 ml Fehling B, boil for few minutes, add 3ml of urine and boil again	Red or Yellow precipitate appears	Glucose is confirmed.

3.	**TEST FOR KETONES** Rothera's test: To 5 ml of urine add solid Ammonium sulphate,saturate it completely, add 2 drops of sodium nitroprusside solution,2 ml of strong Ammonia solution along the sides of the test tube and wait for 10 minutes.	Permanganate colour develops	Ketones like acetone is present.
4.	**TEST FOR BILE SALTS** To 5 ml of urine solution, sprinkle sublimed sulphur powder on the surface.	a) Powder sinks to the bottom b) Powder floats on the surface	Bile salt present. Bile salt absent.
5.	**TEST FOR BILE PIGMENTS** Modified Gmelins test: To 10 ml of urine add 2-3 drops of dil.Hcl, filter it through filter paper,allow it to dry and then put conc.HNO_3 at the apex of the paper \	Colouration on the paper is on the following order-green, blue, violet, red and yellowish-red seen.	Bile pigments is present.
6.	**TEST FOR BLOOD** a) BENZIDINE TEST: Pinch of benzidine powder + 1 ml of glacial acetic acid, shake well for 1 minute + 2 ml urine + few drops of H_2O_2	Green/blue colour due to iron-benzidine formation.	Blood is present.

QUALITATIVE ANALYSIS OF URINE SAMPLE NO:1

SL NO:	TEST	OBSERVATION	INFERENCE
I	**TEST FOR INORGANIC CONSTITUENTS**		
a.	**TEST FOR BICARBONATES:** To 3 ml urine + dil Hcl or dil.H_2SO_4		
b.	**TEST FOR CHLORIDES:** To 5 ml of urine + 1 ml of conc.HNO_3 + 1 ml of $AgNO_3$ solution.		
c.	**TEST FOR PHOSPHATES:** To 3 ml of urine + 3 ml conc.HNO_3 + 3 ml of ammonium molybdate solution and heat to boil.		
d.	**TEST FOR SULPHATES:** To 5 ml urine + 1 ml of conc.Hcl+ 2ml $BaCl_2$ solution.		
e.	**TEST FOR AMMONIA:** a) To 5 ml urine + 2 ml 40% NaOH + boil and hold a litmus paper in the vapour.		
f.	b) Dip a glass rod in phenolphthalein indicator and hold on fumes.		
g.	**TEST FOR CALCIUM:** To 5 ml urine + few drops of NaOH + 1% acetic acid + 2-3 ml of ammonium oxalate solution.		

II.	**TEST FOR ORGANIC CONSTITUENTS**		
a.	**TEST FOR UREA:** a) 3 ml of urine + few drops of alkaline sodium hypobromate solution. b) 5 ml of urine + 4 drops of phenolphthalein + pinchfull of ureas powder (jack bean/soybean meal) and mix. Allow to stand for 5 minutes.		
b.	**TEST FOR URIC ACID:** a) Moisten a strip of filter paper with $AgNO_3$ solution add it a drop o urine. b) 5 ml of urine + 5 drops of benedicts uricacid reagent + 3 gms of anhydrous Na_2CO_3 and mix by shaking.		
c.	**TEST FOR CREATININE:** a) Weyl's test- 5 ml of urine + 5 drops of sodium nitroprusside + 2 ml of 10% NaOH. b) Jaffes test-5 ml of urine + 1 ml of saturated solution of picric acid + 3 gm of anhydrous Na_2CO_3 mix well by shaking.		

III.	**TEST FOR ABNORMAL CONSTITUENTS**		
1.	**TEST FOR PROTEINS:** d) Salphosalicylic acid test: 3 ml urine + sulphosalicylic acid drop by drop e) Heller's nitric acid ring test : 3 ml of conc.HNO_3 + add from side of test tube dropwise urine sample. f) Heat-coagulation test : 5 ml of urine + 2 drops of chlorophenol red, adjust the pH faint by adding 1% aceticacid or 2% Na_2CO_3, boil for 2 minutes.		
2.	**TEST FOR SUGARS:** b) Benedict's test: 5 ml of urine + 5 ml of benedicts reagent, boil for 2 minutes and cool. c) Fehlings test: 2 ml fehling A + 2 ml Fehling B, boil for few minutes, add 3ml of urine and boil again.		

3.	**TEST FOR KETONES** b) Rothera's test: To 5 ml of urine add solid Ammonium sulphate,saturate it completely, add 2 drops of sodium nitroprusside solution,2 ml of strong Ammonia solution along the sides of the test tube and wait for 10 minutes.		
4.	**TEST FOR BILE SALTS** To 5 ml of urine solution, sprinkle sublimed sulphur powder on the surface.		
5.	**TEST FOR BILE PIGMENTS** Modified Gmelins test: To 10 ml of urine add 2-3 drops of dil.Hcl, filter it through filter paper,allow it to dry and then put conc.HNO_3 at the apex of the paper		
6.	**TEST FOR BLOOD** b) BENZIDINE TEST: Pinch of benzidine powder + 1 ml of glacial acetic acid, shake well for 1 minute + 2 ml urine + few drops of H_2O_2 .		