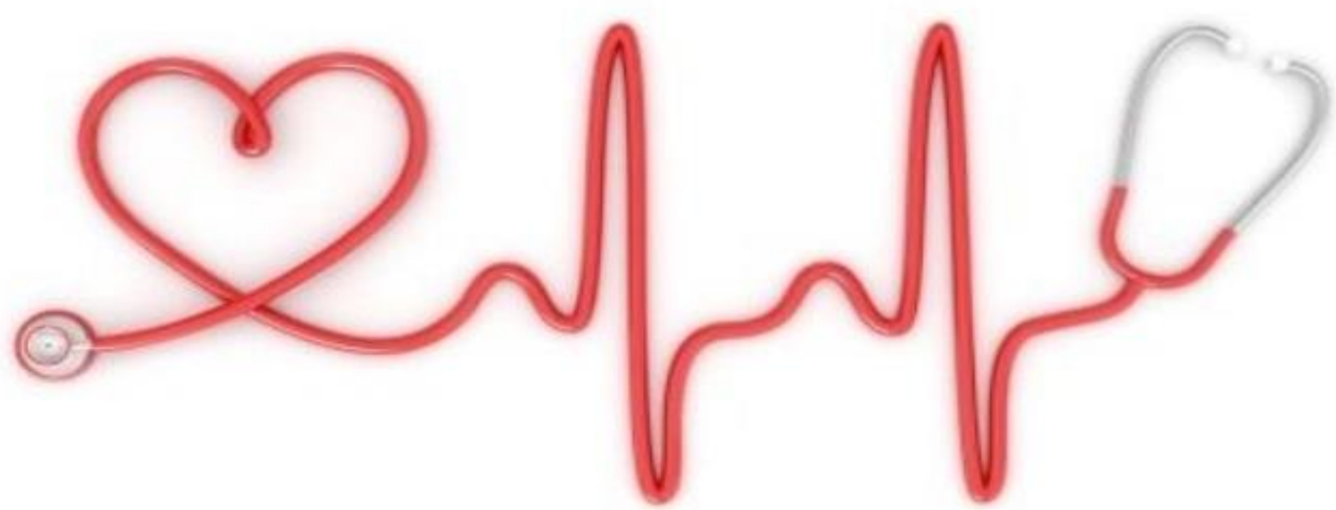
BGS SCIENCE ACADEMY AND RESEARCH CENTRE

(Affiliated to Bengaluru North University)

Jnanagangotri campus, Agalagurki, Chikkaballapura

General Physiology Practical Manual



Name of the student: ______________________________________

I Year, I Semester

Postgraduate Department of Zoology

CONTENTS

Practical – 01 **Date:**

STUDY OF BLOOD PRESSURE DURING REST AND EXERCISE

Aim: To determine the effect of exercise on blood pressure.

Introduction: Blood Pressure (BP) is the pressure exerted by the blood on walls of the blood vessels. Blood pressure is often referred as 'arterial blood pressure' i.e., the pressure in the large arteries delivering blood to body parts such as the brachial artery (in the arm). The systolic pressure is defined as peak pressure in the arteries during the cardiac cycle; the diastolic pressure is the lowest pressure during the resting phase of the cardiac cycle. BP values are universally stated in millimetres of mercury (mmHg). Average blood pressure of a resting healthy adult human being is 120/80 mmHg. BP is measured using the device called Sphygmomanometer.

Working Principle of Sphygmomanometer

The sphygmomanometer cuff is inflated to well above expected systolic pressure. As the valve is opened, cuff pressure slowly decreases. When the cuff's pressure equals the arterial systolic pressure, blood begins to flow past the cuff, creating blood flow turbulence and audible sounds. Using a stethoscope, these sounds are heard and the cuff's pressure is recorded. The blood flow sounds will continue until the cuff's pressure falls below the arterial diastolic pressure. The pressure when the blood flow sounds stop indicates the diastolic pressure.

Protocol for Measurement of BP

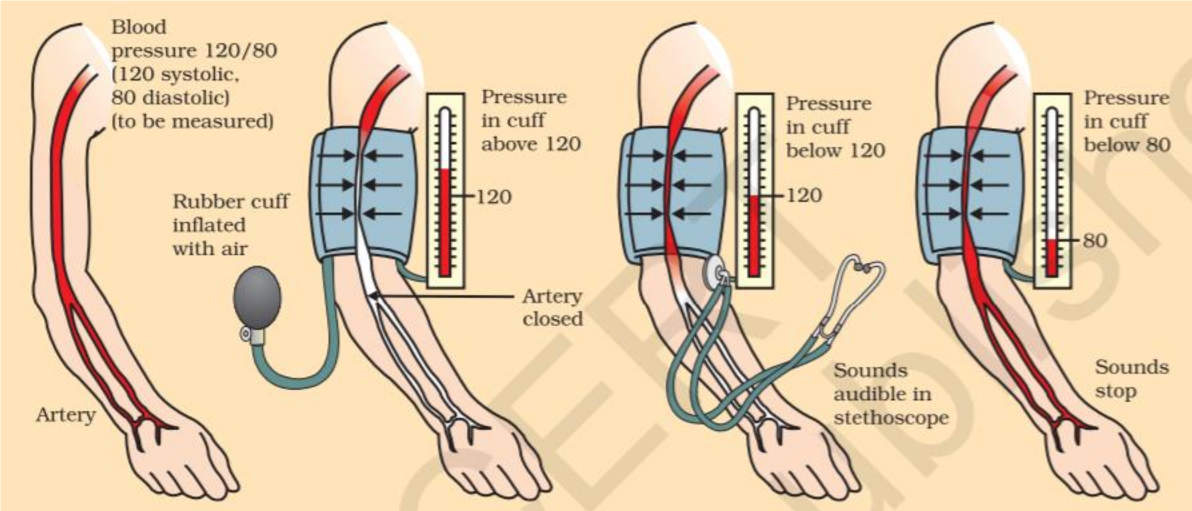
Students blood pressure was recorded while they are resting and they were made to run (exercise) for known distance around 200 meters and immediately after the exercise blood pressure is noted using sphygmomanometer.

- Wrap the cuff around the upper arm with the cuff's lower edge one inch above the antecubital fossa.
- Lightly press the stethoscope's bell over the brachial artery just below the cuff's edge. Some health care workers have difficulty using the bell in the antecubital fossa, so we suggest using the bell or the diaphragm to measure the blood pressure.
- Rapidly inflate the cuff to 180mmHg. Release air from the cuff at a moderate rate (3mm/sec).
- Listen with the stethoscope and simultaneously observe the sphygmomanometer. The first knocking sound is the subject's systolic pressure. When the knocking sound disappears, that is the diastolic pressure (such as 120/80).
- If the subject's pressure is elevated, measure blood pressure two additional times, waiting a few minutes between measurements.

Result:

- Blood pressure of boys at rest = _________________ mmHg
- Blood pressure of boys after exercise = ___________mmHg
- Blood pressure of girls at rest = __________________mmHg
- Blood pressure of girls after exercise = ___________ mmHg

Discussion:



Observation & Calculation

Blood pressure of girls/boys at rest.

Systolic pressure				
Sl. No.	x	x̄	x- x̄ = d	d^2
1				
2				
3				
4				
5				
6				

Diastolic pressure				
Sl. No.	x	x̄	x- x̄ = d	d^2
1				
2				
3				
4				
5				
6				

Mean $(\bar{x}) = \Sigma \frac{fx}{n} = \frac{x1 + x2 + x3 + ...+xn}{n}$

Standard Deviation (SD) = $\sqrt{\frac{\Sigma d2}{n}}$

Standard error = $\frac{SD}{\sqrt{n}}$

Blood pressure of girls/boys after exercise.

Systolic pressure				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

Diastolic pressure				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

Mean $(\bar{x}) = \Sigma \frac{fx}{n} = \frac{x1 + x2 + x3 + ...+xn}{n}$

Standard Deviation (SD) = $\sqrt{\frac{\Sigma d2}{n}}$

Standard error = $\frac{SD}{\sqrt{n}}$

Practical – 02 **Date:**

VISIT TO YOGA CENTRE / DEMONSTRATION BY AN EXPERT

Introduction:

Yoga is essentially a spiritual discipline based on an extremely subtle science which focuses on bringing harmony between mind and body. It is an art and science for healthy living. The word "Yoga" is derived from the Sanskrit root 'yuj' meaning "to join", "to yoke" or "to unite". According to Yogic scriptures, the practice of Yoga leads to the union of an individual consciousness with the universal consciousness.

"Yoga" also refers to an inner science comprising of a variety of methods through which human beings can achieve union between the body and mind to attain self-realisation. The aim of Yoga practice (sādhana) is to overcome all kinds of sufferings that lead to a sense of freedom in every walk of life with holistic health, happiness and harmony.

Brief history and development of Yoga

The science of Yoga has its origin thousands of years ago, long before the first religion or belief systems were born. The seers and sages carried this powerful Yogic science to different parts of the world including Asia, the Middle East, northern Africa and South America. Interestingly, modern scholars have noted and marvelled at the close parallels found between ancient cultures across the globe. However, it was in India that the Yogic system found its fullest expression. Agastya, the saptarishi, who travelled across the Indian subcontinent, crafted this culture around a core Yogic way of life.

Yoga is widely considered as an "immortal cultural outcome" of the Indus Saraswati Valley Civilisation - dating back to 2700 BC - and has proven itself to cater to both material and spiritual upliftment of humanity.

After Patanjali, many sages and Yoga masters contributed greatly for the preservation and development of the field through well documented practices and literature. Yoga has spread all over the world by the teachings of eminent Yoga masters from ancient times to the present date. Today, everybody has conviction about Yoga practices towards prevention of diseases, and promotion of health. Millions of people across the globe have benefitted by the practice of Yoga and the practice of Yoga is blossoming and growing more vibrant with each passing day. Every year 21st June observed as "**International Day of Yoga**".

I. TĀḌĀSANA (Palm Tree Posture) Tāḍa means palm tree or mountain. This asana teaches one to attain stability and firmness and forms the base for all the standing asana.

Technique:

- Stand with feet 2 inches apart.
- Interlock the fingers, and turn the wrist outwards. Now inhale, raise the arms up above your head.
- Raise the heels off the floor and balance on the toes as you raise your arms. Stay in this position for 10-30 seconds.
- Bring the heels down.
- Exhale, release the interlock of the fingers and bring the arms down and come back to standing posture.

Benefits:

- This āsana brings stability in the body, helps to clear up congestion of the spinal nerves and corrects faulty posture.
- Helps to increase height up to a certain age.

A Word of Caution

- Avoid lifting the heals in case of acute cardiac problems, varicose veins and vertigo.

II. VṚKṢĀSANA (The Tree Posture)

Vṛkṣa means tree. The final position of this āsana resembles the shape of a tree, hence the name.

Technique:

- Stand with feet 2 inches apart.
- Focus on a point in front.
- Exhale, hold and bend the right leg then place the foot on the inner side of the left thigh. The heel should be touching the perineum region.
- Inhale and extend the arms up and join the palms together for Namaskar Mudra.
- Stay in the position for 10 to 30 seconds and breathe normally.
- Exhale bring the arms down. Release the right leg and bring it to initial position.
- Repeat this āsana from the left side also.

Benefits

- Helps to improve neuro-muscular coordination, balance, endurance, alertness and concentration.
- It tones up the leg muscles and rejuvenates the ligaments.

A word of caution

- Please avoid this practice in case of arthritis, vertigo and obesity.

III. PĀDA-HASTĀSANA (The Hands to Feet Posture)

Pāda means feet, hasta means hands. Therefore, Pāda Hastāsana means keeping the palms down towards the feet. This is also referred as Uttānāsana.

Technique:

- Stand straight with feet 2 inches apart.
- Inhale slowly and raise the arms up. Stretch up the body from the waist.
- Exhale and bend forward until both palms rest on the ground.
- Stretch the back, to make it straight as much as possible.
- Maintain this final posture for 10-30 seconds with normal breathing.
- Those who are suffering with stiff back should bend according to their capacity.
- Now inhale, come up slowly to the upright position and stretch the arms straight above the head.
- Exhale, slowly return to the starting position in the reverse order.
- Relax in Samasthiti.

Benefits:

- Makes the spine flexible, improves digestion, prevents constipation and menstrual problems.

A word of caution:

- Please avoid this practice in case of cardiac disorders, vertebral and disc disorders, abdominal inflammation, hernia and ulcers, glucoma, myopia, vertigo and during pregnancy.

IV. ARDHA CAKRĀSANA (The Half Wheel Posture)

Ardha means half. Cakra means wheel. In this posture, as the body takes the shape of a half wheel, hence it is called Ardha Cakrāsasna.

Technique

- Support the back at the sides of the waist with the fingers.
- Try to keep the elbows parallel.
- Drop the head backwards stretching the neck muscles.
- As you inhale, bend backwards from the lumbar region; exhale and relax.
- Stay here for 10-30 seconds with normal breathing.
- Inhale and slowly come up.

Benefits

- Ardha Cakrāsana makes the spine flexible and strengthens the spinal nerves. Strengthens the spinal muscles, and improves breathing capacity. Helps in cervical spondylosis.

A word of caution

- Avoid this posture in case of vertigo or a tendency to giddiness.
- Hypertensive patients should bend with care.

V. TRIKONĀSANA (The Triangle Posture)

Trikoṇā means triangle. Tri means three and koṇa means an angle. As the āsana resembles the triangle made by the trunk, arms and legs, hence the name Trikoṇāsana.

Technique

- Stand with your feet with 3 feet apart.
- Inhale slowly raise both the arms sideways upto shoulder level. Turn the right foot towards right side.
- Exhale, slowly bend to the right side and place the right hand just behind the right foot.
- The left arm straight in line the right arm.
- Turn the left palm forward.
- Turn your head and gaze at the tip of the left middle finger.
- Remain in the posture for 10-30 seconds with normal breathing.
- Inhale, slowly come up.
- Repeat the same procedure from the left side.

Benefits

- Prevents flat foot.
- Strengthens calf, thigh and waist muscles.
- Makes the spine flexible, improves lungs capacity.
- Found beneficial in the management of lumber spondylosis.

A word of caution

- Avoid this posture in case of slipped disc, sciatica, and after undergoing abdominal surgery.
- Do not try beyond limits and overdo the lateral stretch. If one cannot touch the feet, one can reach for the knees instead.

VI. VAJRĀSANA (Thunderbolt Posture)

This is considered as one of the meditative postures. While practicing it for meditative purposes, one should close his/her eyes at the final stage. Sthiti: Daṇḍāsana.

Technique:

- Sit with extended legs together, hands by the side of the body, palm resting on the ground, fingers pointing forward.
- Fold the right leg at the knee and place the foot under the right buttock.
- Similarly folding the left leg, place left foot under the left buttock.
- Place both the heels so that the big toes touch each other.
- Position of the buttocks is in the space between the heels.
- Keep both hands-on respective knees.

- Keep the spine erect, gaze in front or close the eyes.
- While returning to the original position, bend a little towards right side, take out your left leg and extend it.
- Similarly extend your right leg and return to the original position.
- Relax in Viśrāmāsana.

Benefits

- This āsana strengthens thigh muscles and calf muscles.
- This āsana is good for digestion.
- It provides firm base to the body and helps to keep the spine healthy.

A word of caution

- Persons suffering from piles should not practise this āsana.
- Those who are suffering from knee pain and ankle injury should avoid this practice.

VII. USṬRĀSANA (Camel Posture)

Uṣṭra means camel. The body in this pose resembles a camel, hence the name.

Technique:

- Sit in Vajrāsana. Bring the knees and the feet about few inches apart and stand on your knees.
- While inhaling bend backward place the right palm on right heel and left palm on left heel and exhale.
- Be careful not to jerk the neck while bending backward.
- In final position, thighs will be vertical to the floor and head tilted backwards.
- Weight of the body should be evenly distributed on the arms and legs.
- Remain in the posture for 10-30 seconds with normal breathing.
- Return with inhalation; sit in Vajrāsana.
- Relax in Viśrāmāsana.

Benefits:

- Uṣṭrāsana is extremely useful for defective eyesight.
- This is useful in relieving back and neck pain.
- It helps to reduce fat over the abdomen and hips.
- It is helpful in digestive problems and cardio-respiratory disorders.

A word of caution:

- Those suffering from heart diseases and hernia should not practice it.

VIII. VAKRĀSANA (The Spinal Twist Posture)

Vakra means twisted. In this āsana, the spine is twisted which has a rejuvenating effect on its functioning.

Technique:

- Bend the right leg and place the right foot beside the left knee.
- Bring the left arm around the right knee and clasp the right big toe or place the palm beside right foot.

- Take the right arm back and keep the palm on the ground with the back straight. Exhale, twist your body to the right.
- Remain in the posture for 10-30 seconds with normal breathing and relax. Inhale take out your hands and exhale to relax.
- Repeat the same on the other side.

Benefits

- Helps to increases flexibility of the spine.
- Helps to overcome constipation, dyspepsia.
- Stimulates pancreas functions and helps in the management of diabetes.

A word of caution

- Please avoid this posture in case of acute back pain, vertebral and disc disorders, after abdominal surgery and during menstruation.

IX. BHUJAṄGĀSANA (The Cobra Posture)

Bhujaṅga means snake or cobra. In this āsana, the body is raised like the hood of the snake, hence the name.

Technique:

- Lie down on your stomach, rest your head on your hands and relax the body.
- Now join your legs and stretch your arms.
- Keep the forehead on the ground.
- Now place your hands just beside the body; keep palms and elbows on the ground.
- As you inhale slowly, lift the head and chest up to navel region without changing in the position of hands. Stay there comfortably. This is called Sarala Bhujaṅgāsana.
- Now come back and place your forehead on the ground.
- Keep your palms besides the chest and raise your elbows where they are.
- Inhale, slowly lift the head and chest up to navel region.
- Keep the elbows parallel and maintain the posture for 10-30 seconds with normal breathing. This is Bhujaṅgāsana.
- Exhale, rest your forehead on the ground, come back to Makarāsana and relax.

Note: Keep the legs firm so that no load or strain is felt on the lumbar spine.

Benefits

- This āsana is best for stress management.
- It reduces abdominal fat and alleviates constipation.
- It also helps to remove backache and bronchial problems.

A word of caution

- Those who have undergone abdominal surgery should avoid this āsana for 2-3months.
- Those who suffer from hernia, ulcers should not practice this āsana.

X. ŚAVĀSANA (The Corpse/ Dead Body Posture)

Sava means dead body. The final position in this āsana resembles a corpse/dead body.

Technique:

- Lie down on your back with arms and legs comfortably apart.
- Palms facing upward, eyes closed.
- Relax the whole body consciously.
- Become aware of natural breath and allow it to become slow and shallow. Remain in the position till you feel refresh and relaxed.

Benefits

- Helps to relieve all kinds of tensions and gives rest to both body and mind.
- Relaxes the whole psycho-physiological system.
- The mind, which is constantly attracted to the outer world, takes a U-turn and moves inwards, thus gradually getting absorbed; as the mind turns quiet and absorbed, the practitioner remains undisturbed by the external environment.
- It is found very beneficial in the management of stress and its consequences.

Surya Namaskar (Sun Salutation)

Surya Namaskar also known as **Sun Salutation** or **Salute to the Sun**, is an all-time favourite of many yogis. It is a very systematic technique which combines the **twelve asanas** in a yoga sequence of 12 steps.

Step 1: Prayer pose – Pranamasana

- Stand at the end of your mat, keep your feet together and distribute your weight on both feet equally.
- Open your chest and just relax your shoulders.
- Breathe in and lift both your arms up from the sides.
- Exhale and bring your palms together in a prayer position in front of your chest.

Step 2: Raised Arm Pose - Hastauttanasana

- Breath in and lift your arms up and back.
- Make sure that your biceps are close to your ears.
- Make effort to stretch your whole body starting from the heels up to the finger tips.

Step 3: Hand to Foot Pose – Hasta Padasana

- Breathing out and bend forward from your waist while keeping your spine straight.
- Exhale and completely bring your hands down to the floor beside your feet.

Step 4: Equestrian Pose – Ashwa Sanchalanasana

- Breath in and push your right leg as far back as possible.
- Bring your right knee to the floor and slowly look up.

Step 5: Stick Pose – Dandasana

- Breath in and take your left leg back
- Bring your whole body in a straight line.

Step 6: Salute with eight parts or points – Ashtanga Namaskara
- Gradually bring your knees down to the floor then exhale.
- Slightly take your hips back and slide forward.
- Relax your chest and chin on the ground.
- Elevate your posterior a little bit.

Step 7: Cobra pose – Bhujangasana
- Slide forward and raise your chest up into the Cobra pose.
- Keep your elbows bent and fixed in this pose.
- Keep your shoulders away from your ears.
- Slowly look up.

Step 8: Mountain pose – Parvatasana
- Breath out and lift your hips as well as your tail bone up.
- Put your chest downwards to create an inverted V pose.

Step 9: Equestrian pose – Ashwa Sanchalanasana
- Breath in and bring your right foot forward in between the two hands.
- Bring your left knee down to the ground.
- Press your hips down then look up.

Step 10: Hand to Foot Pose – Hasta Padasana
- Breathing out and bend forward from your waist while keeping your spine straight.
- Exhale and completely bring your hands down to the floor beside your feet.

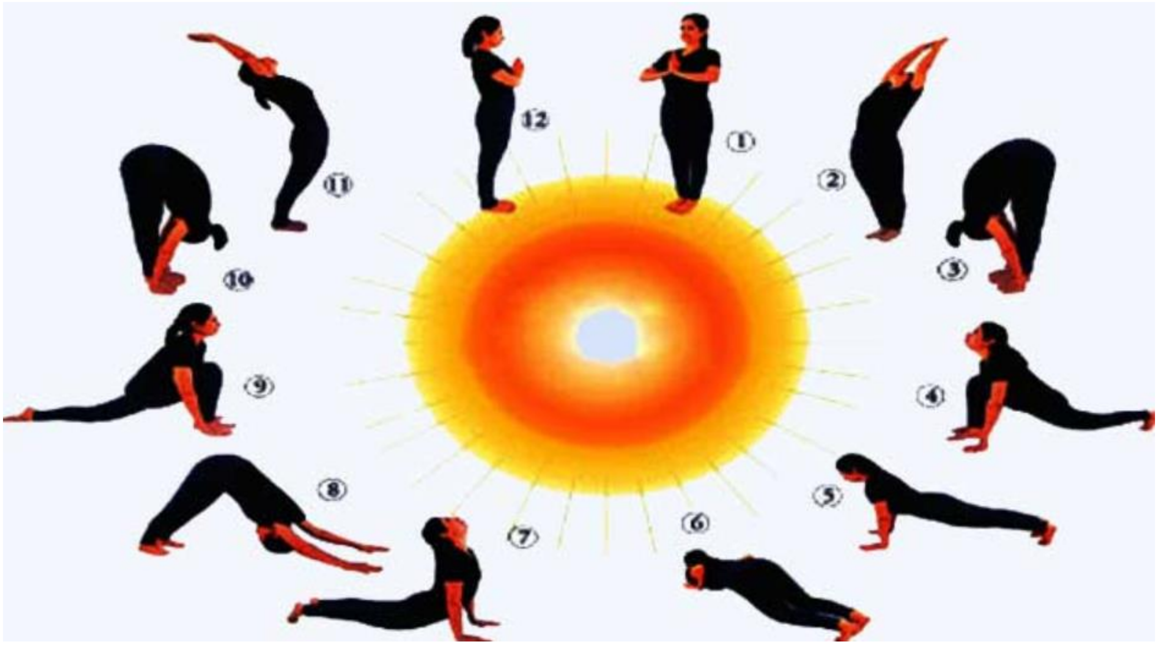
Step 11: Raised Arm Pose – Hastauttanasana

- Breath in and lift your arms up and back.
- Make sure that your biceps are close to your ears.
- Make effort to stretch your whole body starting from the heels up to the finger tips.

Step 12: Standing Mountain pose – Tadasana
- Exhale and first straighten your body.
- Bring your arms down.

Benefits:
- Good for Weight Management.
- Cognitive Skills Booster.
- The Salute to the Sun can help balance the three doshas - Pita, Kapha and Vata
- It can help improve the blood circulations so as to prevent blood pressure issues and palpitation.

- Surya Namaskar is beneficial for the lungs and can help decrease any chances of getting tuberculosis.



Practical – 03 **Date:**

Effect of Exercise on Breathing Rate, Pulse Rate and Blood Lactate

Part A: Breathing Rate

Introduction: Breathing rate is defined as the number of breaths (inhalation and exhalation cycles) recorded within a set amount of time (typically 60 seconds). Breathing rate is also known as respiration rate, ventilation frequency, respiration frequency, pulmonary ventilation rate, breathing frequency. Normal respiration rate is termed as '**Eupnea**', increased or rapid respiratory rate than normal is termed as '**Tachypnea**' and decreased respiratory rate than normal is termed as '**Bradypnea**'.

Principle: As exercise commence pulmonary ventilation increases in direct proportion to the intensity and metabolic needs of the exercise ventilation increases to meet the demands by

- Increasing tidal volume which refers to the quantity of air that is inhaled and exhaled with every breath.
- Increased respiration rate leads to the fast supply of oxygen to body for the metabolic needs, which in turn helps in the release of energy to required tissue.

Procedure: Respiration rate is measured when the students are at rest which involves counting number of breaths for one minute. Students breathing rate was recorded when they were at rest and after exercise. Students are subjected to aerobic exercise by running for known distance. Immediately after the exercise breathing rate is recorded.

Result:

Breathing rate of girls at rest ________________breaths per minute

Breathing rate of girls after exercise __________breaths per minute

Breathing rate of boys at rest _______________breaths per minute

Breathing rate of boys after exercise __________breaths per minute

Discussion:

Observations and Calculations

Breathing rate of girls/boys.

At rest				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

After Exercise				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

Mean $(\bar{x}) = \Sigma \frac{fx}{n} = \frac{x1 + x2 + x3 + ...+xn}{n}$

Standard Deviation (SD) = $\sqrt{\frac{\Sigma d2}{n}}$

Standard error = $\frac{SD}{\sqrt{n}}$

Part B: Pulse Rate

Introduction: Pulse rate or heart rate or pulse is the number of times heart beats per minute i.e., the heart pumps lowest amount of blood that a person needs to survive in a relaxed state and in absence of illness. Heart rate is normally between 60-100 beats per minute. Normal heart rate varies from person to person which an important heart health gauge. Common pulse points include the carotid artery of the neck, the brachial artery inside the elbow, and the radial artery in the wrist. A lower heart rate is also common for people who get a lot of physical activity in athletics. The heart muscles are in better condition and doesn't need to work hard to maintain a steady beat.

Principle: Pulse, rhythmic expansion of the arteries resulting from passage of successive surges of blood, produced by continuing contractions of the heart. The arteries resemble elastic tubes, and at each contraction of the heart, 30 to 60 g of blood are forced into the already-filled vessels. The consequent distension passes along the arterial system at a rate of about 7 m a second until it reaches the capillaries, in which it is lost because of peripheral resistance to blood flow and lack of elasticity in the vessel walls.

Protocol to Measure Pulse Rate:

To check your pulse at your wrist, place two fingers between the bone and the tendon over your radial artery which is located on the thumb side of your wrist. When you feel your pulse, count the number of beats in 60 seconds.

Students pulse rate was recorded at rest and they were made to run for a known distance for physical exercise. Again, pulse rate was measured immediately after the exercise.

Result: Pulse rate of girls at rest __________ beats per minute

Pulse rate of girls after exercise __________ beats per minute

Pulse rate of boys at rest __________ beats per minute

Pulse rate of boys after exercise __________ beats per minute

Discussion:

Observations and Calculations

Pulse rate of girls/boys.

At rest				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

After Exercise				
Sl. No.	x	$\bar{x}$	$x - \bar{x} = d$	d^2
1				
2				
3				
4				
5				
6				

$$\text{Mean } (\bar{x}) = \Sigma \frac{fx}{n} = \frac{x1 + x2 + x3 + \ldots\ldots + xn}{n}$$

$$\text{Standard Deviation (SD)} = \sqrt{\frac{\Sigma d2}{n}}$$

$$\text{Standard error} = \frac{SD}{\sqrt{n}}$$

Practical – 03 (C) **Date:**

Part C: Effect of Exercise on Blood Lactate of Rat

Aim: To estimate the concentration of blood lactate in exercised and resting rat.

Introduction:

Blood lactate concentration is one of the most often measured parameters during clinical exercise testing as well as during performance of athletes, because measurement of elevated blood lactate may be used to evaluate and underlaying pathology during stress tests (coronary artery disease, chronic airway obstruction, chronic renal failure, metabolic impairment) and also to prescribe appropriate exercise to different populations, In response to progressive increment in the exercise the blood lactate concentrations increases gradually first and then rapidly as the exercise become more intense.

Principle:

Blood lactate is ordinarily determined by conversion to acetaldehyde which is then measured by titrimetric or colorimetric method. In obtaining blood for lactate determination precautions must be observed against conversion of blood glucose to lactate on standing. The use of fluoride as anticoagulant prevents glycolysis. If oxalate or heparin are used the protein free filtrate should be prepared as soon as possible after drawing the blood.

The glucose and other interfering material of protein free blood filtrate is prepared by Vanslyke – Salkowski method of treatment with copper sulphate and calcium hydroxide and aliquot of the resulting solutions is heated with concentrated sulphuric acid to convert lactate to acetaldehyde which is then determined by the reaction with Para hydroxy diphenyl in the presence of copper ions.

Reagents Required:

i. **20% Copper sulphate:** Dissolve 20g of copper sulphate $5H_2O$ in 100ml of distilled water.
ii. **4% Copper sulphate:** Dissolve 1g of copper sulphate $5H_2O$ in 100ml of distilled water.
iii. **Calcium hydroxide powder:** 1gram approximately
iv. **Concentrated sulphuric acid**
v. **Para hydroxy di phenyl reagent:** Dissolve 1.5g of Para hydroxy diphenyl in 10ml of 5% NaOH solution, place with little water by warming and stirring and dilute to 100 ml with water. Store in brown colored bottle fitted with stopper and this reagent and it is stable for many months.
vi. **Standard lactate:** Dissolve 0.213g pure dry lithium lactate in about 100ml of water in a 1L volumetric flask and add about 1ml concentrated

sulphuric acid, dilute to mark with distilled water and mix this solution. It contains 1mg of lactate in 5ml and is stable at 0-5^{0}C.

vii. **Working standard:** Dilute 5ml of stock to 100ml, this solution contains 0.01 mg of lactate per ml and is best when prepared freshly.

Procedure:

- Deproteinize the blood sample (whole blood) with either tungstic acid, TCA or zinc hydroxide at 1:10 dilution.
- Transfer 2ml of protein free filtrate representing 0.2 ml of blood to a centrifuged tube graduated at 10ml.
- In a second tube 5ml of standard lactate solution containing 0.01mg of lactic acid per ml.
- In a third tube take 5ml distilled water and it acts as a blank, and serves to control the small amount of color yielded by the reagents alone.
- To each tube add 1ml of 20% $CuSO_4$ solution and dilute to 10ml with distilled water.
- Add 1g of $Ca(OH)_2$ to each tube and shake vigorously until the solids are uniformly dispersed allowed to stand for half an hour, repeating the shaking at least once in every 5 minutes.
- Centrifuge at 2000 rpm for 10 minutes (down the ppt) and take 1 ml of supernatant to clean and dry test tube.
- To each test tube add 0.05ml of 4% $CuSO_4$ solution, followed by 6ml of concentrated sulphuric acid **(drop by drop),** mixing the contents during the addition.
- Place the test tubes in boiling water bath for 5 minutes, then transfer the test tubes to cold water.
- After cooling add 0.1ml of Para hydroxy diphenyl reagent to each tube, the reagent precipitates out, mix well and place the tubes in a beaker of water at 30^o C for 30 minutes. Re-disperse the precipitated reagent at least once during this period.
- Place the tubes in vigorously boiling water for 90seconds, remove and cool in cold water at room temperature.
- Record the OD in colorimeter at 560 nm.

Result: The amount of blood lactate found in controlled rat is __________ mg/ml

The amount of blood lactate found in exercised rat is __________ mg/ml

Discussion:

Observations and Calculations:

Sl. No.	Optical Density of sample	OD at 560 nm
1	OD of blank	
2	OD of control sample	
3	OD of experimental sample	
4	OD of standard	

I. **For control sample:**

$$\frac{OD\ of\ sample\ (control) \times Concentration\ of\ Standard}{OD\ of\ standard}$$

_________________________ mg lactic acid/100ml of blood

II. **For experimental sample:**

$$\frac{OD\ of\ sample\ (experimental) \times Concentration\ of\ Standard}{OD\ of\ standard}$$

_________________________ mg lactic acid/100ml of blood

Practical – 04 **Date:**

Active Transport of Glucose Through Intestinal Wall of Vertebrate

Aim: To study the active transport of glucose through intestinal wall of rat.

Principle: Low concentration of glucose is injected into small intestine and thereafter suspended into the high concentration of glucose for an hour. The transportation of molecules occurs from the region of its lower concentration to the region of higher concentration with expenditure of energy (ATP).

Reagents Required:

1. Ringer solution: Mix 960 ml of 0.124 M NaCl, 20 ml of 0.154 M KCl and 20 ml of 0.11M $CaCl_3$
2. 1% Glucose: 1g of glucose dissolved in 100ml distilled water.
3. 0.1% Glucose: 0.1g of glucose dissolved in 100ml distilled water.
4. Concentrated Sulphuric Acid.

Procedure:

- Dissect out the small intestine of rat and wash inside and outside with ringer solution.
- Tie one end of the intestine and inject 0.1% of glucose and tie other end of the glucose then measure the length between the two knots.
- Suspend the intestine in 1% of glucose and allow it for one hour.
- After one hour, take out the contents of intestine into test tube and add 5ml of concentrated H_2SO_4.
- Mix well and heat in water bath for ten minutes.
- Take optical density in a colorimeter at 540nm.

Preparation of Blank: For 1 ml of distilled water add 5 ml of concentrated H_2SO_4

Result: The rate of active transport of glucose across the small intestinal wall of the rat is ___________ mg cm^{-1} $hour^{-1}$

Discussion:

Observation and Calculation:

Length of intestine :

Amount of 0.1% injected into intestine : 1 ml

Concentration of glucose injected :

Time allowed for active transport : 1 hour

Standard optical density (OD) :

Experimental OD : 'x' =

Experimental OD corresponds to (y) : $\frac{Conc.of\ std. \times x}{OD\ of\ Std.} =$

Amount of glucose transported : 1- y =

Rate of active transport(α) : $\frac{Amount\ of\ glucose\ transported}{length\ of\ intestine}$

: _____ mg of glucose cm^{-1} $hour^{-1}$

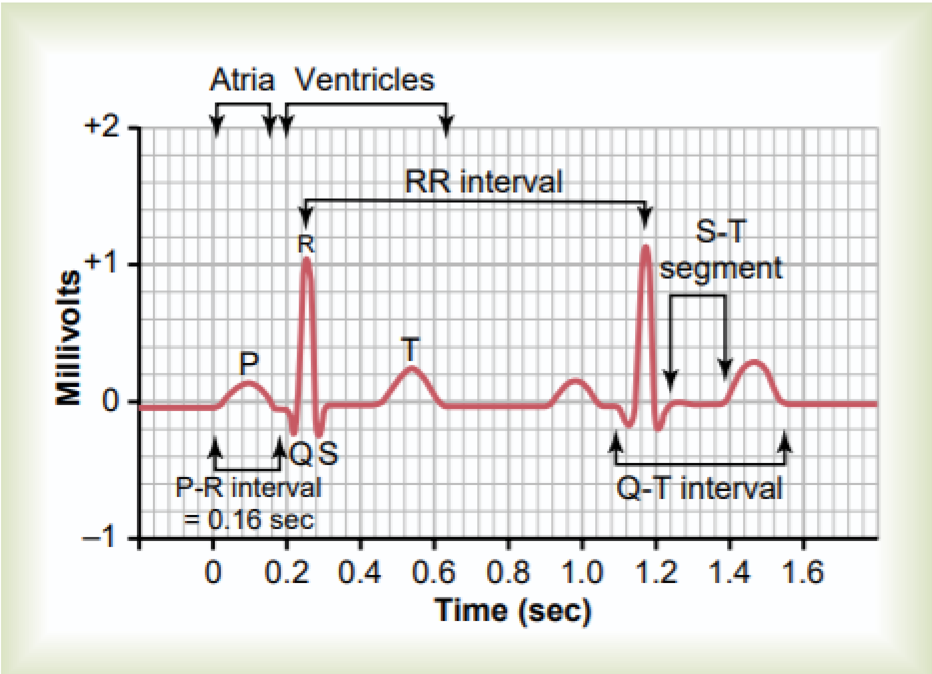
*For one hour (If time allowed for 30 mins) : α×2 mg of glucose cm^{-1} $hour^{-1}$

Practical – 05 **Date:**

Electrocardiogram and its interpretation

Electrocardiogram (ECG) is the recording of the electrical activity of the heart muscle fibers during each heart beat as a graph. The instrument used to record the changes is an electrocardiograph. The graph can show the heart's rate and rhythm, as well as provide indirect evidence of blood flow to the heart muscle.

Each cardiac cycle produces three distinct waves, designated as P, QRS and T. The different waves that comprise the ECG represent the sequence of depolarization and repolarization of the atria and ventricle.



Normal Electrocardiogram

P Wave: It's a small and upward deflection in the ECG and it represents atrial depolarization, which spreads from the Sino Atrial (SA) node throughout the atria, and is usually 80-100 milliseconds in duration.

QRS Complex: It indicates the onset of the contraction of the ventricles. It represents rapid ventricular depolarization, as the action potential spreads into the ventricles.

Q Wave: It is the first downward (negative) deflection after P wave and corresponds to depolarization of intraventricular system.

R Wave: R wave is the upward (positive) deflection after Q wave and it is produced by depolarization of main mass of ventricles.

S Wave: It represents last phase of ventricular depolarization as the base of heart.

T Wave: It indicates ventricular repolarization and occurs just as the ventricles are starting to relax. The T wave is smaller and wider than the QRS complex because repolarization occurs more slowly than depolarization.

PR interval: PR interval includes the PR segment. PR Segment represents the time taken by the AV node to conduct the impulse. The PR interval shortens at high heart rates (due to exercise) and increases at lower heart rates (during sleep).

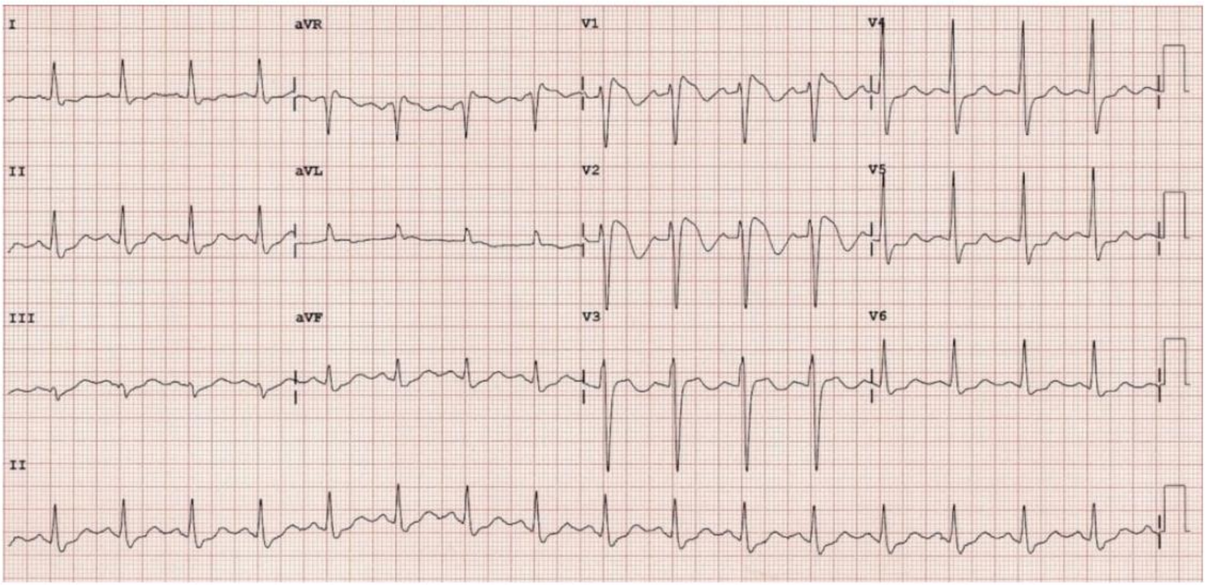
ST Segment: It represents the time gap between the depolarization and repolarization in the ventricles. The segment lies between QRS and T.

QT Interval: It is measured from the beginning of the QRS complex to the end of the T-wave and represents the time between the activation of electrical activity in the ventricles and their return to the resting state.

Brugada Syndrome

Brugada syndrome is the genetic disease that is characterised by abnormal electrocardiogram. Findings and increased risk of sudden cardiac arrest approximately 20% of the cases of Brugada syndrome have been shown to be associated with the mutations in the gene that encodes for the sodium ion channel in the cell membrane of the muscle cells of the heart (myocytes). The gene named SCN5A is located on the short arm of the third chromosome (3P21).

This results in the transmural and epicardial dispersion of repolarization. The transmural dispersion underlies S-T segment elevation and the development of a window across a ventricular wall, recording of ventricular fibrillation (VF) or polymorphic ventricular tachycardia (VT).



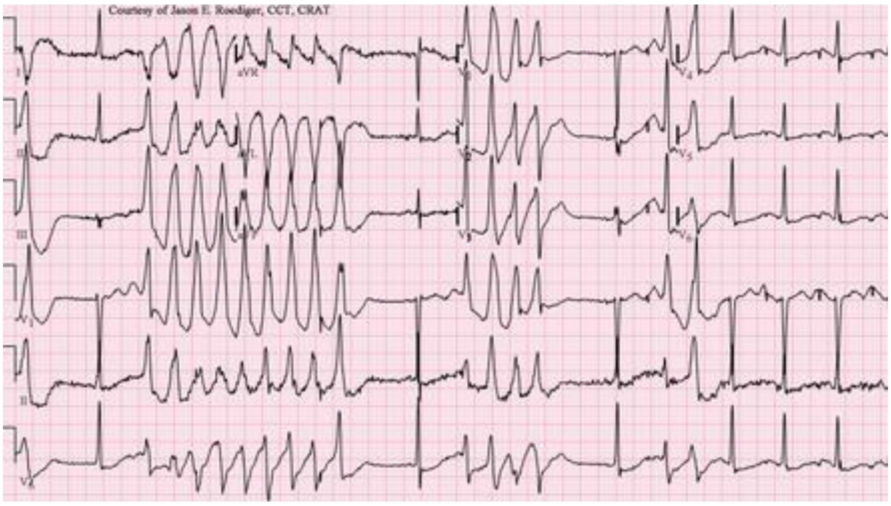
Brugada Syndrome

Torsades De Pointes

Torsades de pointes describes a condition of the heart. It is a polymorphic ventricular tachycardia that exhibits distinct characteristics on the electrocardiogram.

Drastic rotation of the heart's electrical axis prolonged, QT interval, long QT segment may not be present in the short-coupled variant of Torsade de pointes triggered by a pre-ventricular contraction (R-on-T Phenomena).

Long QT syndrome can either be inherited as congenital mutations of ion channels carrying the cardiac impulse action potential or acquired as a result of drugs that blocks these cardiac ion currents.



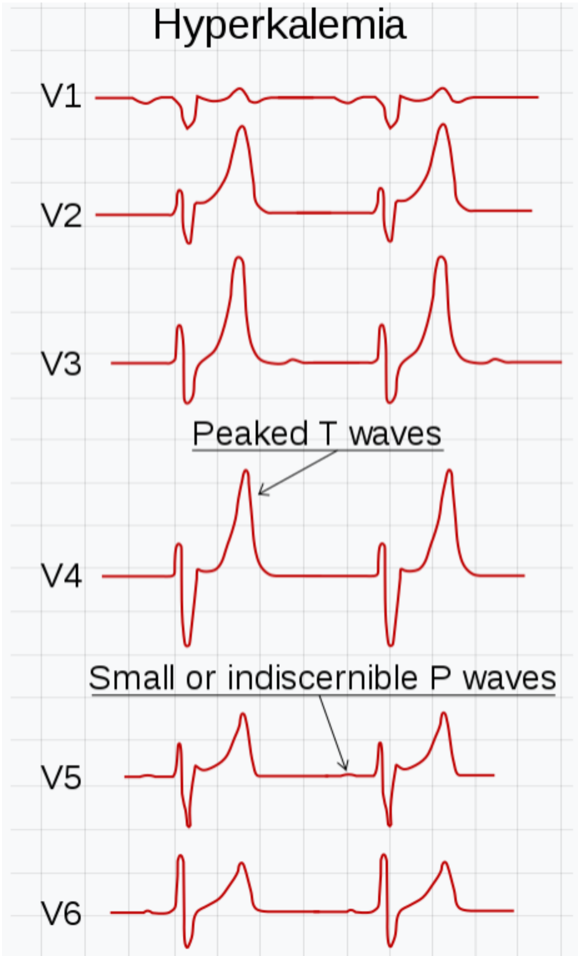
Torsades de pointes

Hyperkalaemia

Recording of the peaked T wave (early), Prolonged P-R interval, flattening of P wave, widening of QRS complex (increased risk of arrhythmia), Sine waves (fusion of QRS and T wave) and Ventricular arrhythmia in ECG can be seen.

The symptoms of elevated potassium in the body are nonspecific and the condition is usually discovered on blood tests performed for other reasons. Extreme hyperkalaemia is a medical emergency due to the risk of potentially fatal abnormal heart rhythms.

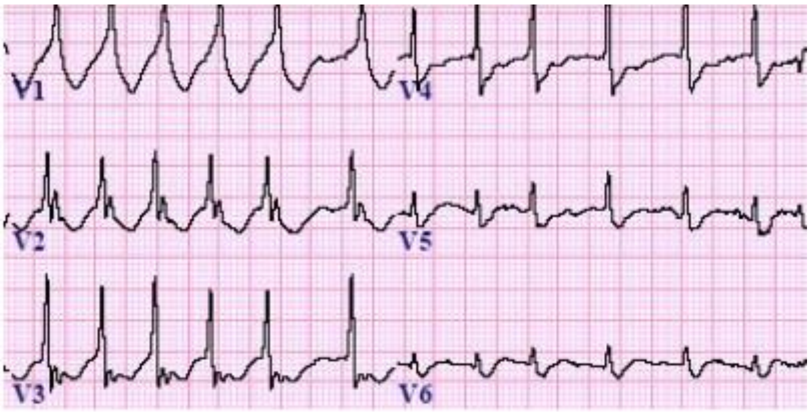
Temporary treatment given can be given to reduce the risk of complications but removal of the excess potassium by stimulating urine production or through dialysis is usually necessary.



Ventricular Tachycardia

Ventricular tachycardia is a rapid cardiac arrythmia originating in the ventricles. Sometimes people tolerate ventricular tachycardia with only minimal symptoms but more typically arrythmia produces significant palpitations, severe lightness, syncope (loss of consciousness) or even cardiac arrest and sudden death most of the time. Ventricular tachycardia develops as a result of an underlaying cardiac disorder that produces damage to the ventricular tachycardia muscle most commonly coronary artery disease or heart failure and scarred cardiac muscle tends to produce tiny electrical circuits with in the heart muscle. Circuits that can cause reentrant tachycardia.

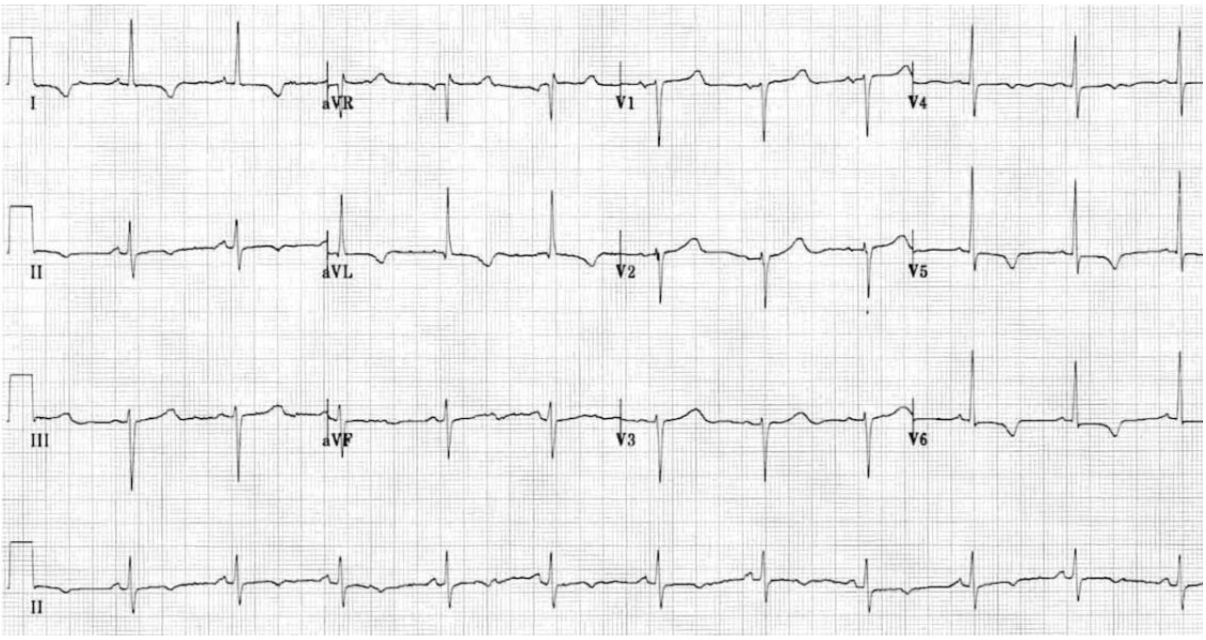
Ventricular tachycardia refers to a wide QRS complex heart rhythm that is, a QRS duration beyond 120 milliseconds, originating in the ventricles at a rate of greater than 100 beats per minute. This can be hemodynamically unstable, causing severe hypotension.



Anterolateral/Inferior Ischemia

Anterolateral ischemia occurs when blood flow to anterolateral side of the heart is reduced, preventing the heart muscle from receiving enough oxygen. The reduced blood flow is usually the result of a partial or complete blockage of heart's arteries (coronary arteries)

Anterolateral ischemia ECG recordings shows significantly the ST segment depression and T wave flattening or inversion. ST depression can be either up sloping, down sloping, or horizontal. T wave inversion may be considered to be evidence of myocardial ischaemia if, at least 1 mm deep and present in ≥ 2 continuous leads that have dominant R waves.



Anterolateral/Inferior Ischemia

Practical – 06 **Date:**

Measurement of Ascorbic Acid in blood

Aim: To estimate the amount of ascorbic acid in given sample of blood.

Principle: Ascorbic acid is a powerful reducing agent which reduces 2,6 dichlorophenol indophenol (DCPIP) dye and itself getting oxidized to dihydroxy ascorbic acid. Titration is carried in presence of metaphosphoric acid acetic acid solution in order to inhibit ascorbic acid solution (catalyzed by certain metallic ions) by inactivating enzymes to precipitate and liberate protein bound ascorbic acid. The rate of reduction of the dye is directly proportional to the amount of ascorbic acid liberated.

Reagents Required:

i. 2,6 Dichlorophenol indophenol dye: 42 mg of sodium bicarbonate and 53 mg of 2,6 dichlorophenol indophenol powder dissolved in 100 ml of distilled water and make up to 200ml with distilled water.
ii. Metaphosphoric acid acetic acid solution: 15 grams of metaphosphoric acid is dissolved in 40 ml of glacial acetic acid and add 450 ml of distilled water.
iii. Standard ascorbic acid: 100 mg of crystalline ascorbic acid dissolved in 100 ml metaphosphoric acid acetic acid solution.
iv. 10% Ethylenediamine tetraacetic acid (EDTA): 10g EDTA in 100ml distilled water.
v. 5% Hematocrit (hct): To 1ml blood add 9ml of 10% EDTA.

Procedure:

Part A: Estimation of Ascorbic Acid

Take 1ml of 5% of hematocrit blood and make up to 5ml with metaphosphoric acid acetic acid solution. Incubate samples at room temperature for 5 minutes. Centrifuge and collect the supernatant make up the volume to 20ml by adding metaphosphoric acid acetic solution. Appearance of pale pink color is the end point.

Part B: Standardization of Indophenol Dye

10ml of standard ascorbic acid solution is diluted to 25ml with metaphosphoric acid acetic acid solution. Take 5ml of above solution and titrate against 2,6 Dichlorophenol indophenol dye. Appearance of pale pink color is the end point.

Result: The amount of ascorbic acid present in given sample is _________ mg/ml of blood.

Discussion:

Observations and Calculation:

Part A: Estimation of Ascorbic Acid

Burette reading	Trial 1	Trial 2	Trial 3
Final Burette Reading			
Initial Burette Reading			
Volume of 2,6 DCPIP rundown			

Volume of 2,6 DCPIP rundown= α =________ ml

Part B: Standardization of Indophenol dye

Burette reading	Trial 1	Trial 2	Trial 3
Final Burette Reading			
Initial Burette Reading			
Volume of 2,6 DCPIP rundown			

Volume of 2,6 DCPIP rundown= β = _________ml

- 25ml of metaphosphoric acid acetic acid contains 10 mg of ascorbic acid.
- 5ml of metaphosphoric acid has = 'x' = $\frac{10}{25} \times 5$ = 2mg of ascorbic acid.
- 'α' ml of dye is required to neutralize the 2mg of ascorbic acid.
- 'β' ml of dye is required to neutralize = 'y' = $2 \times \frac{\beta}{\alpha}$ =_____ ml
- 5ml of sample contains 'y' mg
- 20ml of sample contains = $\frac{y \times 20}{5}$ = 'z' mg
- 'z' mg of ascorbic acid present in 1ml of blood.

Practical – 07 **Date:**

Determination of excretory products in ureotelic ammonotelic, and uricotelic animals.

Aim: To estimate the excretory products in ureotelic ammonotelic, and uricotelic animals.

I. Estimation of urea

Principle: This method is to detect the presence of urea by indirect means. The urea present in the blood is treated with the urease enzyme to obtain ammonium carbonate, since the basis for urea determination of essentially the analysis for ammonium reduced by the action of enzyme. The result should be carried for the performed or pre-existing ammonia present in the sample is estimated calorimetrically with Nessler's reagent.

Reagents Required:

1. Nessler's reagent
2. Urease solution: 100 mg of purified urease in 100 ml distilled water.
3. Sodium tungstate (10%)
4. Urea

Procedure:

I. Agglutination of blood: Draw 1ml of blood, add 8ml distilled water 0.5ml of 10% sodium tungstate incubate for 10 minutes at room temperature filter and collect the filtrate.
II. In first test tube take 1ml of distilled water, in second test tube take 1ml of standard solution and in third test tube 1ml of blood filtrate.
III. Add few drops of urease solution and allow the reaction in closed tube at room temperature for 25 minutes.
IV. After 25 minutes of incubation place the test tubes in boiling water bath for 5 minutes to inactivate the enzyme.
V. To the above mixture add 0.5ml of Nessler's reagent (reddish brown/brick colour appears).
VI. Take OD at 580 nm in calorimeter.

Note: Take separate reading for with and without urease solution.

Preparation of standard: 3215 mg /3.215 g of pure dry urea in water and dilute to 500ml distilled water and add few drops of chloroform as a preservative.

Result: The amount of urea present in the given sample ___________ mg/ml

Observation and calculation:

Optical density at 580 nm

Blank	Standard	Sample with urease	Sample without urease

Concentration of urea in sample= $\frac{(OD\ of\ sample\ with\ urease - OD\ of\ sample\ without\ urease)}{OD\ of\ standard} \times concentration\ of\ standard$

=

II. Estimation of ammonia

Principle: Nessler's reagent gives a characteristic reddish-brown colour and intensity of colour is proportional to the amount of ammonia in sample.

Reagents Required:

1. Nessler's reagent
2. Ammonia solution

Procedure: To 5ml of water sample add 0.5ml of Nessler's reagent. Reddish brown colour obtained. Take OD at 480nm against blank.

Preparation of blank: 5ml distilled water and 0.5ml of Nessler's reagent.

Preparation of standard: 1ml concentrated ammonia solution made up to 500ml with distilled water.

Result: The amount of ammonia in the given sample ________ mg/ml

Observation and calculation:

Blank	Standard	Sample

Concentration of ammonia= $\frac{OD\ of\ sample}{OD\ of\ standard} \times concentration\ of\ standard$

III. <u>Estimation of Uric Acid:</u>

Principle: Uric acid is determined photometrically after adding photo tungstic acid and a best which gives blue colour. The intensity of this colour is directly proportional to amount of uric acid present.

Reagents required:

1. 14% sodium carbonate
2. Uric acid reagent

Procedure: To 1ml of filtrate add 0.25ml of 14% sodium carbonate followed by 0.25ml of uric acid reagent. Read OD at 660-780nm against blank.

Preparation of blank: 1ml of distilled water add 0.25ml of 14% sodium carbonate followed by 0.25ml of uric acid reagent.

Preparation of standard: 100mg of uric acid dissolved in 50ml of distilled water and transferred it in to a 100ml. 13g of sodium carbonate is dissolved in 35ml of distilled water and the solution is warmed at 50^{0}C. This solution is added to the flask, 10ml of 1N H_2SO_4 is added gradually and the solution is diluted to 100ml with distilled water.

Result: Amount of uric acid present in the given sample __________mg/ml.

Observation and calculation:

Blank	Standard	Sample

Concentration of uric acid= $\frac{OD\ of\ sample}{OD\ of\ standard} \times concentration\ of\ standard$

Discussion:

*****