MANUAL

FOR

MEDICAL PHYSIOLOGY PRACTICAL

Name …….

Registration Number ……

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DEPARTMENT OF PHYSIOLOGY

FACULTY OF MEDICINE

UNIVERSITY OF JAFFNA

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This manual has been developed by the academic staffs of the Department. The Departmental technicians have helped in typing and designing this Manuel. The cover page is designed by Mr. N. Thileepan, office of the Dean.

Introduction

GENERAL OBJECTIVES IN PHYSIOLOGY

The aim of the course is to develop basic understanding of the functions of the body and their applications in management of patients and to develop skills in assessing the functions of systems of the body and basic clinical examination. At the end of the course the students should be able to,

- Describe the basic principles of homeostasis, water and electrolyte balance, acid base balance, energy balance and temperature regulation.
- Describe the role of various systems of the body, how they function, the mechanisms that regulate them and the factors that alter the functions.
- Outline how pathological factors interfere with the functions of these systems and how altered functions of these systems cause disease.
- Describe the physiological basis of various tests used to assess the functions of these systems and interpret the results obtained.
- Mention the names of common chemical agents that alter the functions of these systems and outline the mechanism of their actions.
- Investigate blood for haemoglobin concentration, red cell count, white cell count, differential count, bleeding time, clotting time, blood groups and packed cell volume.
- Measure body fat, measure blood pressure, lung volumes, pulmonary ventilation, concentration of oxygen and carbon dioxide in alveolar air, metabolic rate, body temperature, urine flow and specific gravity of urine
- Feel arterial pulse and recognize rate, regularity and volume of the pulse, identify normal heart sounds, identify waves and intervals in normal E.C.G, record respiratory movements, perform cardiorespiratory resuscitation and examine basic sensory and motor functions and special sensations.
- Having attained the knowledge and skills mentioned above, the student should view man as a whole organism and not a collection of systems, apply the knowledge and skills in understanding and managing patient problems and keep on continued study of Physiology.

The teaching learning activities include lecture discussions, practical classes, tutorials and clinical demonstrations. Lecture discussions will be delivered by the departmental staff where students are informed of the topics well in time and are expected to read up based on the objectives given to them at the beginning of the course as a book. Practical classes will be conducted in the laboratory with the aim of developing basic clinical skills related to Physiology and to demonstrate important physiological principles. Tutorials will be in different forms such as free oral question-answer sessions, answer writing sessions, sessions for students to clear their doubts and so on as requested by the students. Clinical demonstrations are conducted to illustrate clinical significance of pre-clinical learning by bringing selected patients from the Teaching Hospital or showing relevant video clips and demonstrating the clinical application of the basic sciences at the end of each section. All these activities will be interactive encouraging student participation and performance instead of simple delivery of information. The Clinical Departments of the Faculty will be conducting the clinical demonstrations and, if need arises, consultants from the Teaching Hospital will be invited as Visiting Lecturers. In addition, video shows on functions of various systems are shown to illustrate their structure and function.

Further, there will be formative evaluations at the end of or during the course of each section or system. The marks of in-course assessments conducted at the end of each term will be given to students and the answers will be discussed with the students. The students are given detailed objectives for the course in physiology and guides for each practical class developed by the department as teaching material.

AIMS OF THE PHYSIOLOGY PRACTICAL COURSE

The students are expected to benefit from the practical classes in the following ways:

1. Learn and acquire skills.
2. Acquire an aptitude for careful observation.
3. Familiarize with nomograms.
4. Gain skill in designing simple experiments.
5. Familiarize with simple statistical concepts.
6. Gain skills in recording an experiments, tabulating and condensing data.
7. Learn to draw valid conclusions from available data.
8. Practice writing a report
9. Practice looking up, indexing, and abstracting journals and tracing the literature references on a particular subject.
10. Gain knowledge of concepts of validity, reliability, precision and errors in measurements.
11. Supplement to oral classes.
12. Apply Physiological learning to health and community problems.

INTRODUCTION TO EXPERIMENTAL PHYSIOLOGY

Careful observation is the back bone of scientific method and so one of the aims of conducting experiments is to acquire an aptitude for careful observation. Often this depends simply upon intelligent use of the sensory organs. But observation calls for proficiency in special techniques frequently and therefore some of the experiments will be to learn techniques. These provide proficiency in techniques for subsequent experiments. The skill in practical work will grow in the process of learning these techniques and this skill is valuable in all aspects of clinical practice and research.

Observation yield information only when properly analyzed. Thus the second object of the training is to learn how to make logical inferences from observations. All facts learnt in any science course are conclusions drawn from the results of many experiments. The most important aim of the course in Experimental Physiology is to understand how knowledge is acquired from scientific observations and to verify certain facts given in the text books. It is not possible to perform experiments to confirm and verify all the theoretical information that are obtained from lectures and text books. In the course of Experimental Physiology, a select number of experiments will be done which will give some understanding of the scientific methods as applied to different aspects of Physiology

If the course in Experimental Physiology is to achieve these objectives, it is imperative that theoretical background of each practical is obtained before doing the experiment. Therefore, students are expected to have read about that day's experiment before coming to the class.

The practical work in laboratory is only a part of an experiment. An equally important part is to record it properly. Another aim of the course in Experimental Physiology is to learn proper documentation of the procedures and observations and to be able to comment on them. Students can clarify their own thinking while recording their observations, inference and comments; and if this is done properly, students will gain far more from the experiments than if they stopped with performing the experiments.

A good record will also help to review the experiments before practical examinations.

Some of the principles to be followed in writing up your record are:

1. The write-up must contain all the information necessary for somebody else to repeat the experiment if necessary.
2. The record is essentially an account of what was done and what was observed and so need not elaborate on theoretical aspects.
3. Legibility, neatness and brevity are three virtues.

THE FORM OF RECORDING

An accepted form of recording is given below:

1	Aim:	Write the aim of the experiment in one sentence.
2	Principles:	State in one or two sentences how the aim is achieved
3	Apparatus:	List the apparatus required, Describe fully any new or item preferably with a diagram.
4	Procedure:	Describe briefly the exact procedure followed, in order.
5	Precautions:	Every experiment will usually have a few points which have to be specially taken care of, Mention these specifically.
6	Observations:	This is the most important part of the write up. Always an open and critical mind. Describe fully what you have observed? If possible tabulate your observations in order. Give diagrams when desirable, with adequate labels.
7	Calculations:	If any.
8	Discussion:	Here you can write the inferences from your observations. Also make any relevant comments on the limitations of the Experimental techniques and any alterations or additions that you would like to make. Specially discuss any unusual observation of your findings. Avoid extensive theoretical discussions. It is a good practice to make your discussion no longer than the account of your observations.

Note:

1. Students are expected to fill the gaps given in this manual to record the experiment while they are in the class and submit for correction at the end of the class.
2. When data of many students is to be entered and analyzed, students are expected to provide such data to be fed to the computer and complete data will be printed and distributed to students.

Experiment 1

MEASUREMENT OF HEIGHT, WEIGHT, BODY SURFACE AREA, BODY FAT, WAIST & HIP CIRCUMFERENCE

Measurement of height

Height is one of the parameters that indicate the size of the body. The height, when studied along with other parameters, gives valuable information: for example, it indicates the rate of growth when studied with age of a child; with weight gives body mass index and so on.

Method:

Measure your height with a centimeter scale.

Exercise:

a. Define the correct posture for measuring the height.

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Describe the precautions to be taken when measuring height.

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Measurement of weight

Weight is another useful parameter of the size of the body. As variables such as metabolic rate, energy expenditure, and nutrient requirements are related to body weight, they are often expressed as per unit body weight.

Method:

Measure the weight using a kilogram scale.

Exercise:

a. Explain the factors that affect the measurement of the weight.

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Describe the precautions to be taken when measuring weight.

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Measurement of the body surface area

The surface area has been found to correlate well with many physiological parameters such as cardiac output and metabolic rate. In common practice these parameters are expressed as per unit surface area.

Method:

Direct measurement of surface area is very difficult and time consuming and hence not suitable for routine measurement. This can be determined indirectly from a nomogram using height and weight.

Exercise:

Determine the surface area.

Measurement of Body Fat.

Fat is found in the body in two main forms: structural fat and stored fat. Structural fat is relatively small amount and is in proportion to the mass of the tissues. Stored fat is found in adipose tissue which is seen in specific areas. The amount of stored fat differs among individuals.

The best method to measure the body fat accurately is to analyze the body chemically. Since this method is not possible in live animals, indirect methods are employed. Measurements based on body density, body water, or body potassium are laborious and usually applied for measurements on small number of subjects for research purposes. An easy method which is accurate enough for routine measurement is to predict the fat content from skin-fold thickness.

Instrument:

Harpenden skin-fold calipers.

Method:

The subject sits on a stool comfortably. At the sites of measurement, skin-fold is pinched up firmly between the thumb and forefinger and pulled away slightly from the underlying tissue before applying the calipers. The calipers are applied so that the foot plate is vertical to the surface. The calipers exert constant pressure at varying opening of the jaws. The width of the opening is read off a scale incorporated in the apparatus. The reading is taken when the needle in the scale stabilizes soon after the application. All measurements are taken on the right side of the body. At least four measurements are made in each standard site and the mean is calculated.

The standard sites for measurements are:

Biceps: Over the mid-point of the muscle belly with the arm resting supinated on the subject's thigh.

Triceps: Over the mid-point of the muscle belly, mid-way between the olecranon and the tip of the acromian with the upper arm hanging vertically.

Subscapular: Just below the tip of the inferior angle of the scapula, the arm hanging vertically, at an angle of about 45° to the vertical.

Suprailiac: Just above the iliac crest in the mid-axillary line.

Exercise:

Measure the skin fold of a subject, enter in this table and determine the total body fat.

	Biceps	Triceps	Subscapular	Suprailiac
Measurement 1				
Measurement 2				
Measurement 3				
Measurement 4				
Mean				

The total thickness of the skin in all four sites: …….

Percentage weight of fat by age and sex, read in the table below……..

A less accurate method is to predict body fat from the triceps skin-fold thickness. In this method the thickness of the skin over the mid triceps is measured and the percentage of fat read from appropriate table.

SKINFOLD THICKNESS AND BODY FAT CONTENT
TABLE FOR MALES AND FEMALES

Total Skin fold (mm)	Males (age in years)					Females (age in years)			
	17-29	30-39	40-49	50+		16-29	30-39	40-49	50+
20	8.1	12.2	12.2	12.6		14.1	17.0	19.8	21.4
30	12.9	16.2	17.7	18.6		19.5	21.8	24.5	26.6
40	16.4	19.2	21.4	22.9		23.4	25.5	28.2	30.3
50	19.0	21.5	24.6	26.5		26.5	28.2	31.0	33.4
60	21.2	23.5	27.1	29.2		29.1	30.6	33.2	35.7
70	23.1	25.1	29.3	31.6		31.2	32.5	35.0	37.7
80	24.8	26.6	31.2	33.8		33.1	34.3	36.7	39.6
90	26.2	27.8	33.0	35.8		34.8	35.8	38.3	41.2
100	27.6	29.0	34.4	37.4		36.4	37.2	39.7	42.6
110	28.8	30.1	35.8	39.0		37.8	38.6	41.0	43.9
120	30.0	31.1	37.0	40.4		39.0	39.6	42.0	45.1
130	31.0	31.9	38.2	41.8		40.2	40.6	43.0	46.2
140	32.0	32.7	39.2	43.0		41.3	41.6	44.0	47.2
150	32.9	33.5	40.2	44.1		42.3	42.6	45.0	48.2
160	33.7	34.3	41.2	45.1		43.3	43.6	45.8	49.2
170	34.5	34.8	42.0	46.1		44.1	44.4	46.8	50.0
180	35.3	-	-	-		-	45.2	47.4	50.8
190	35.9	-	-	-		-	45.9	48.2	51.6
200	-	-	-	-		-	46.5	48.8	52.4
210	-	-	-	-		-	-	49.4	53.0

Estimation of Body Mass Index (BMI)

BMI is an objective scientific measure of height to weight ratio which correlates well with body fat.
The following formula is used to calculate BMI

$$BMI == \frac{\text{Weight (Kg)}}{\text{Height}^2\ (m^2)}$$

It can also be read from the nomogram.

Measurement of Waist and Hip Circumferences

Waist circumference is considered as a good index for abdominal obesity. Waist – Hip ratio and waist – height ratio are considered as more reliable parameters to indicate metabolic syndrome.

Waist circumference: Make the subject to stand. Measure the waist circumference midway between the uppermost border of the iliac crest and the lower border of the costal margin. Place the non-elastic measuring tape around the abdomen at the level of this mid-point. Make sure the tape is snug, but does not compress the skin and it is parallel to the floor. Take the measurement at the end of expiration. In overweight people it may be difficult to accurately palpate those bony landmarks .In this case place the tape at the level of the umbilicus.

Hip Circumference: Make the subject to stand with feet together and weight evenly distribute on both feet. Place the non-elastic measuring tape around the maximum extension of the buttocks and take the measurement. Make sure the tape is applied snugly and it is horizontal.

Observations:

Name [males]	Height (cm)	Weight (Kg)	Waist Circum ference (cm)	Hip Circum ference (cm)	Total Skin Fold (mm)	Body Fat (%)	BMI Kg/m^2	Surface Area (m^2)	Waist / Hip ratio	Waist / Height Ratio
Name [Females]	Height (cm)	Weight (Kg)	Waist Circum ference (cm)	Hip Circum ference (cm)	Total Skin Fold (mm)	Body Fat (%)	BMI Kg/m^2	Surface Area (m^2)	Waist / Hip ratio	Waist / Height Ratio

Interpretation:

Classification of weight status according to BMI in Asian Adults:

Category	**BMI (kg/m^2)**	**Risk of co-morbidities**
Underweight	< 18.4	Risk of clinical problems related to malnutrition
Normal (healthy)	18.5 – 22.9	Average
Overweight	>23.0	
At risk	23.0 – 24.9	Mild
Obese class I	25.0 – 29.9	Moderate
Obese class II	> 30.0	Severe

World Health Organization cut-off points and risk of metabolic complications:

Indicator	**Cut-off points**	**Risk of metabolic complications**
Waist circumference	>94 cm (M); >80 cm (W)	increased
Waist circumference	>102 cm (M); >88 cm (W)	Substantially increased
Waist–hip ratio	≥0.90 (M); ≥0.85 (W)	Substantially increased

M, men; W, women

Cut off values for Waist circumference in South Asians according to International Diabetes Federation criteria:

Male -90 cm, Female -80cm

Waist - Height Ratio for Sri Lankans:

≥ 0.5 – increased obesity associated with metabolic risks.

Exercise:

Define "Mean"

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Define "SD"

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Discussion:

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Experiment 2

OSMOTIC FRAGILITY AND PERMEABILITY PROPERTIES OF RED BLOOD CELLS

When cells are placed in hypotonic solutions water enters the cell due to osmosis and causes the cells to swell. When cells are distended beyond a limit, the cell membrane is stretched and the contents of the cell leak out. This effect can be easily detected when red cells are studied because haemoglobin leaks out and forms a clear pink solution (haemolysis).

Demonstration

Observe the steps in drawing blood form a volunteer (venipuncture). Note the chemical used to prevent blood clotting (anticoagulant) and the care taken in mixing the anticoagulant with blood to prevent mechanical haemolysis.

Anticoagulant used:……

The following solutions are prepared:

a) sodium chloride: serial dilution from 0 to 0.9%,
b) glucose: serial dilution from 0 to 5%,
c) urea: serial dilution from 0 to 2% and
d) 0.9% NaCl with Soap solution (1 ml).

These solutions [10 ml] are taken in two sets of test tubes and placed in racks.

Two drops of blood are added to each test tube. One set is kept as it is for observation and the other set centrifuged and kept.

Precautions:

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Exercise

1. Record the concentration of each solution where haemolysis begins and the concentration where haemolysis is complete.

Sodium Chloride	beginning……..	Complete……..
Glucose	beginning……..	Complete……..
Urea	beginning……..	Complete……..

Observation in Soap solution……..

2. Explain the reason for haemolysis.

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3. Explain the factors that affect the fragility of red cells.

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4. Explain the differences of fragility of RBC in different solutions:
   a) Sodium Chloride (NaCl) : M.W = 58.5
   b) Glucose ($C_6 H_{12} O_6$) : M.W = 180
   c) Urea ($CO(NH_2)_2$) : M.W = 60

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(Note: Haemolysis of normal red cells in saline begins at 0.5% and complete at 0.3%).

Blood Work

SAMPLING OF BLOOD

Blood samples will be collected for experiments from the subjects themselves. Most of the experiments would require only a drop of blood which could be obtained conveniently through a puncture at the tip of a finger. When more blood is needed, it is collected by puncturing a vein by a needle and syringing the required blood. These methods are described below.

Finger Puncture

When a finger is punctured, blood in the capillaries will ooze out. This blood should be collected and utilized quickly, before clotting as there are no anticoagulants added, sometimes special heparinized capillary tubes may be used for special investigations. From infants blood sample may be collected conveniently from the heel or the big toe.

Method:

1. Sterilize the skin thoroughly over and around the site to be punctured with a cotton pad soaked with suitable disinfectant (70% alcohol).
2. Wipe the area with dry, sterile gauze pad.
3. Puncture the skin with a sterile lancet. Do a quick, single and sufficiently deep stroke to puncture to allow free flow of blood. Flow of blood could be enhanced by gentle pressure at the proximal part of the finger to obstruct venous return. Squeezing or milking the finger will dilute the blood with tissue fluid.
4. Allow the blood to flow freely and discard the first drop by wiping it away with a dry gauze pad.
5. Obtain the required amount of blood directly using appropriate instrument, pipette or slide.
6. Leave the punctured site undisturbed for the bleeding to stop on its own and if it continues to bleed, cover the area with clean gauze and apply gentle pressure until bleeding stops.

Venipuncture

Venous blood can be conveniently obtained from the veins in the bend of the elbow. A tourniquet applied above the site of collection facilitates blood collection by obstructing the venous return while permitting arterial flow into the site. Other sites may be selected to collect blood from children and dehydrated or collapsed patients. Syringe of appropriate size could be used. The needle should be sterile, sharp and not smaller than 20 gauge in diameter.

Method**:**

1. Inspect the forearm and identify the vein to be punctured.

2. Sterilize the skin over and around the site to be punctured with a cotton pad soaked with suitable disinfectant (surgical spirit- 70% alcohol)

3. Wipe the area with dry, sterile gauze pad.

4. Prepare the syringe and needle carefully following aseptic techniques. Make sure that there is no air in the syringe.

5. Puncture the skin, with the needle attached to the syringe, just closer to the vein [not above the vein] and push the needle for about 2 – 3 mm under the skin.

6. Pull the skin and the needle over the vein and gently push the needle into the vein, maintaining gentle and steady traction of the plunger. As the vein is thin walled, this has to be done carefully to prevent puncturing the opposite wall of the vein and pushing the needle into the tissue beyond the vein. The gentle traction of the plunger will indicate the first puncture into the vein by the flow of blood into the syringe.

7. Fix the syringe and draw the necessary amount of blood.

8. Release the tourniquet first and then place dry gauze over the puncture site, exert a gentle pressure and draw the needle out. Ask the subject to hold the gauze for about five minutes to avoid oozing of blood from the puncture site.

Experiment B1a

MEASURMENT OF ERYTHROCYTE SEDIMENTATION RATE (ESR)

When anti-coagulated blood is allowed to stand, the red cells sediment gradually. The rate of sedimentation depends on the viscosity of the blood and the ratio of the mass to surface area of the red cells. The main determinants of the viscosity of blood are concentration of cells and the composition and amount of plasma proteins. The main determinant of the mass to surface ratio is rouleaux formation.

Equipment:

Westergren tube (recommended by the International Committee for Standardization in Haematology), sedimentation tube holder

Anticoagulant:

Trisodium Citrate dihydrate solution – 0.11 M (3.8%)

Method:

Blood is obtained by venipuncture and 1.6 ml blood mixed with 0.4 ml of anticoagulant (4 volumes of blood and 1 volume of anticoagulant). The Westergren tube is dipped in and the blood drawn to zero mark. The lower end is sealed by plasticene clay. The tube is then placed vertically in the holder for one hour.

Precautions:

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Exercise:

a) Read the length of the clear plasma on top of the tubes in mm (ESR):

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b) Describe the factors that affect the sedimentation of the erythrocyte.

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c) Explain the differences of ESR between males and females.

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d) Describe the use of ESR in clinical practice.

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Experiment B1b

DETERMINATION OF THE PACKED CELL VOLUME (PCV)

When anti-coagulated blood is centrifuged in a tube, the cells are packed in the lower end due to centrifugal force. This separates the cells from the plasma and gives the percentage of the cells in blood. The PCV can be determined for a sample of blood obtained either from a vein (venipuncture) or capillaries (finger prick).

Instruments:

Venous blood: Wintrobe tube, Pasteur pipette and Centrifuge

Capillary blood: Heparinized capillary tubes and Micro-centrifuge

Anticoagulant:

Potassium Oxalate crystals - for venous blood.

(EDTA also can be used)

PCV of venous blood: - Demonstration

Venipuncture is performed to collect 5 ml. of blood which is transferred to a test tube containing a pinch of oxalate crystals mixture. The blood is mixed with anticoagulant by rolling the tube between the palms of both hands. Blood is taken in the Pasteur pipette and introduced into the Wintrobe tube up to the mark ten (10) without introducing air bubble. This is achieved by dipping the pipette to the bottom of the Wintrobe tube and drawing the tip upwards as the blood is ejected into the tube. The tube with blood is centrifuged at 3000 r.p.m. for 15 minutes.

Precautions:

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Results:

PCV of male:……..

PCV of female:…….

Thickness of the Buffy Coat:……

Explain why the red and white cells are separated:

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PCV of capillary blood: - (done by all students)

Perform finger pick and collect blood into a heparinized glass capillary tube to about 75% of its length without any air bubble. Cover the end, through which blood was collected, by a finger and hold the open end on to a source of heat (spirit lamp) in order to seal the tip without heating the blood. Once the tip is sealed keep the tube in a groove, the sealed end facing outwards, in the micro centrifuge and note the number. The centrifuge will be switched on when sufficient number of capillary tubes are loaded and centrifuged for five minutes. After centrifugation, take your tube and read the percentage of packed cells with the help of the Micro-haematocrit reader (instruction for using the reader is found on the back of the reader).

Precautions:

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Observations:

	Name [Males]	PCV (%)	Name[Females]	PCV (%)
1				
2				
3				
	Mean			
	SD			

Discussion:

a. Describe the advantages and disadvantages of both methods.

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b. Describe the use of PCV in clinical practice.

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Experiment B2

WHITE BLOOD CELL COUNT

The blood contains non nucleated red blood cells and nucleated white blood cells. The white cells are far less in number and are larger than red cells. Therefore the technique of counting blood cells can be learnt easily with white cells. But it will be necessary to destroy the red cells and to stain the white cells for easy identification (as they are colorless) and counting.

Instruments:

The White Cell Pipette

The white cell pipette consists of a thick walled capillary tube which is expanded at one point to from a bulb. The tube between the tip and the bulb is divided into ten equal parts; the fifth division is labeled as 0.5 and the tenth as 1.0. The beginning of the capillary beyond the bulb is marked 11. This means that the volume of the bulb is ten times the volume of capillary tube of the first part. This does not represent any known volume but only proportion. If blood is taken up to the mark 0.5 and diluting fluid drawn up to 11, the blood would have been diluted 20 times (1 in 20). This is because the fluid in the capillary would not contain any blood as any form of mixing is unlikely to force the blood back into the capillary tube. Mixing up of the blood in the bulb is promoted by a white bead placed in it.

Haemocytometer (Counting Chamber)

The Improved Neubaur Counting Chamber is widely used. The counting chamber is a special slide with H shaped system of troughs extending right across its middle in such a way that the troughs enclose two platforms with counting area. The platforms are exactly 0.1 mm below the ridges on either side. A number of small squares, in an area of 3 mm wide and 3 mm long are engraved at the center of each platform. This area contains 9 large squares, 1 sq. mm each. The four large squares in the four corners are used to count white cells; each of these squares is divided into 16 small squares. The square at the center is used for counting red cells; this square is divided 25 medium sized squares (0.2 mm x 0.2 mm) and each of these squares is further divided into 16 squares (0.05 mm x 0.05 mm). When a special cover-slip is placed across the ridges above the platforms and the diluted blood is charged into the space between the cover- slip and the platform, the volume of the fluid contained in any one of the large square would be 0.1 mm^3 (1 x 1 x 0.1).

Exercise

Place the counting chamber under low powers (x 4 and x10) of the microscope and study the squares in the counting area.

WBC Diluting Fluid

Turk's solution is used. It is a 3% solution of acetic acid (for destruction of red cells) with trace of Gentian Violet (for staining the white cells).

Method:

Take some diluting fluid in a watch glass and keep it on the table. Attach the rubber tubing (with a mouth piece) to the WBC pipette. Make a finger prick and wipe out the first drop and allow a second drop to from. Hold the pipette horizontally and draw blood in the pipette up to 0.5 mark. Wipe any blood on the exterior of the pipette tip and insert the pipette vertically and draw the diluting fluid up to 11 mark. Draw the diluting fluid carefully and do not exceed the mark because it cannot be reversed. Close the tip of the pipette with the right index finger and remove the rubber tube. Cover the other end of the pipette by the right thumb so that the pipette is held in the right hand such as its both ends are closed. Shake the pipette thoroughly for about one minute. (Now the pipette contains blood diluted 20 times and haemolysed).

Place the counting chamber on the table with the cover-slip in place. Discard two or three drops from the pipette in order to eliminate blood-less fluid in the stem of the pipette. Then, carefully controlling the escape of the fluid with a fingertip applied to the Upper end of the pipette, allow a small drop to from at the tip of the pipette. Apply this drop to the opening between the cover-slip and the platform. (The counting chamber will be charged by the surface tension pulling the fluid inwards. This prevents any air entering the counting area, flooding with excess fluid and spilling over the cover-slip). Examine the counting area under the microscope for general distribution of cells. If uneven distribution is seen, clean the counting chamber and re-charge the counting chamber.

Place the counting chamber under the microscope and identify the engraved lines under the lowest magnification (x 4). Identify the WBC counting areas and move one counting area to the center of the field. Turn to the next magnification (x 10) and start counting the cells systematically. The small squares are there to avoid recounting the same cell or missing any cell; start from the top left square and move across the last square then come down to the next row and so on; in order to avoid re-counting the cells on the lines, adopt a convention to ignore cells touching the upper and left line and to include cells touching the lower and right lines. Count the cells in all four WBC counting areas.

Calculation:

Let the total number of cells counted in all 4 areas be C

The area of one large square = 1 sq.mm.

The depth of the film of fluid = 1/10 mm.

The volume of fluid in all 4 squares = 4 x 1/10 = 0.4 cu.mm.

Assume the number of cells in all areas = C

Number of cells in 1 cu. mm. diluted blood = C/0.4

The dilution is 1 in 20.

Therefore, Cells in 1 cu. mm. undiluted blood = $\frac{Cx20}{0.4} = 50C$

Precautions:

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Observations:

	Name [Males]	**WBC Count (cells/cu.mm)**	Name[Females]	**WBC Count (cells/cu.mm)**
1				
2				
3				
	Mean			
	SD			

Explain the possible errors that could arise in obtaining and diluting blood, due to uneven distribution of cells in the counting chamber, due to mechanical causes and from other sources:

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Discussion:

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Experiment B3

RED BLOOD CELL COUNT

The principle and procedure are similar to leukocyte count with a few modifications

Instruments

The red cell pipette:

The red cell pipette consists of a thick walled capillary tube which is expanded at one point to form a bulb. The tube between the tip and the bulb is divided into ten equal parts the fifth division is labeled as 0.5 and the tenth as 1.0. The beginning of the capillary beyond the bulb is marked 101. If blood is taken up to the mark 0.5 and diluting fluid drawn up to 101, the blood would have been diluted 200 times (1 in 200). This is because the fluid in the capillary would not contain any blood because any form of mixing is unlikely to force the blood back into the capillary tube. Mixing up of the blood in the bulb is promoted by a red bead placed in it.

Haemocytometer (Counting Chamber):

The Improved Neubauer Counting Chamber, described with white cell count is widely used. Of the nine large squares of the counting area, the square at the center is used to count red cells; this square is divided into 25 medium sized squares (0.2mm x 0.2mm). Each of the medium squares is further divided into 16 small squares. Red cells are counted in the four medium sized squares at the four corners and the one at the center. The volume of the fluid contained in one of the medium sized square would be 0.004 mm^3 (0.2 x 0.2 x 0.1).

Exercise:

Place the counting chamber under the microscope and study the red cell counting area on the platform.

RBC Diluting Fluid:

The solution contains 1 volume of 40% formaldehyde (formalin) in 100 volumes of 3% trisodium citrate. The formalin acts as preservative and citrate as anticoagulant.

Method:

Draw blood in the pipette up to 0.5 mark and diluting fluid up to 101 mark and mix well. Charge the counting chamber, place it under the microscope and identify the engraved lines under the lowest magnification (marked "x4"). Identify the RBC counting areas under next magnification (marked "x10") and turn to the next magnification ("x40") and count the cells systematically; the small squares are there to avoid re-counting the same cell or missing any cell; start from the top left square and move across to the last square; then come down to the next row and so on; in order to avoid re-counting the cells on the lines, adopt a convention to ignore cells touching the upper and

left line and to include cells touching the lower and tight lines. Count the cells in all five **RBC** counting areas.

Calculation:

The volume of the fluid in medium square = 0.004 sq.mm.
Volume of fluid in all 5 squares = 5 x 0.004 cu.mm
= 0.02 cu.mm.

Let the total number of cells counted in all 5 areas be C
Number of cells in 1 cu.mm diluted blood = $\frac{C}{0.02}$
= 50C

The dilution is 1 in 200.
Cells in 1 cu.mm. undiluted blood = 50 x 200C
= 10,000C

Precautions:

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Observations:

	Name [Males]	**RBC Count (cells/cu.mm)**	Name[Females]	**RBC Count (cells/cu.mm)**
1				
2				
3				
4				
	Mean			
	SD			

Explain the possible errors that could arise in obtaining and diluting blood, due to uneven distribution of cells in the counting chamber, due to mechanical causes and from other sources:

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Discussion:

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Experiment B4a

MEASUREMENT OF THE HAEMOGLOBIN CONCENTRATION

The amount of haemoglobin in unit volume of blood determines the capacity to transport oxygen. The haemoglobin concentration can be determined accurately by clinical methods but they are laborious and time consuming. There are many easy methods to measure it on the basis of the color index.

Demonstration:

Colorimetric method

Principle:

Drabkin's solution contains Potassium Ferricyanide and Potassium cyanide. When it is added to the blood, hemoglobin is changed into Methemoglobin in the presence of Potassium ferricyanide. This Methemoglobin reacts with Potassium cyanide and form Cyanmethemoglobin which gives a purple colour. Intensity of this colour can be measured at 540 nm. This is proportional to the total hemoglobin concentration.

Instruments: Anti coagulated tube, Drabkin's reagent, Spectrophotometer.

Method:

1. Add 0.5 ml citrate into a graded test tube and add 4.5 ml blood (9 volumes of blood into 1 volume of citrate).
2. Make 1: 250 dilution of blood: Drabkin's reagent and leave for 10 -15 minutes.
3. Measure the Hemoglobin concentration at 540 nm in a spectrophotometer.

Observation:

Heamoglobin concentration:…….

Practical:

Students should employ two methods to measure the haemoglobin concentration of your blood. One is Talqvist method which measures the colour of oxygenated haemoglobin; and the other is Sahli's method which measures the colour of reduced haemoglobin. The haemoglobin is reduced to acid haematin by reaction with hydrochloric acid.

Equipments:

Sahli's haemoglobinometer.
Haemoglobin pipette
Talqvist Chart

Reagents:

Decinormal hydrochloric acid (N/10 HCl)

Methods:

Talqvist method

Make a finger puncture, collect a drop of blood on the standard blotting paper and keep it for one or two minutes until it dries and the surface loses the shiny appearance. Compare the colour chart and determine the haemoglobin concentration (100% = 14.5 g/100 ml) and enter the result on the board.

Sahli's method

Take the hydrochloric acid in the Sahli's tube to the mark 4. Collect 20 µl (mm^3) of blood in the haemoglobin pipette from a finger puncture. While collecting, make sure to hold the pipette horizontally. Do not allow air bubble while drawing blood and use a teat to help in gentle suction. Wipe any blood on the tip of the pipette with a filter paper. If the blood has gone above the mark in the pipette, bring it down by blotting with the filter paper. Dip the tip of the pipette in the acid in the Sahli's tube, near the bottom of the tube, and gently blow the blood out into the tube and wash any residual blood in the tube by sucking and flushing the acid. Mix the blood with acid by rolling the glass rod between the fingers (and not by up and down strokes) and wait until the reaction between the acid and haemoglobin is complete.

Place the tube in Sahli's comparator and add distil water or acid little by little to dilute until the colour matches the standard colour tubes. When the colour is close to the standard add drop by drop and record the level every time. If the dilution exceeds the limit, it cannot be reversed and it will be necessary to start all over again. The haemoglobin concentration is indicated by level of the fluid in the tube as gram percent and percentage of normal. Enter the results on the board.

Describe the Precautions to be taken:

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Observations:

	Name [Males]	Sahli's Method		Talqvist Method	Name[Females]	Sahli's Method		Talqvist Method
		g%	%	%		g%	%	g%
1								
2								
3								
	Mean							
	SD							

Discussion:

a) Describe the advantages and disadvantages of the methods of determination of haemoglobin concentration.

b) Describe the use of haemoglobin concentration in clinical practice.

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Experiment B4b

HAEMATOLOGICAL INDICESS

Demonstration: measurement with auto analyzer.

Calculation:

Calculate the following using the PCV (p%), Hb % (hg/100ml) and RBC count (r x 10^6 cells / cu.mm) measured in this and previous classes.

Mean Corpuscular Volume (MCV)

The volume of a cell is calculated by dividing the volume of cells in 1000 ml blood by the number of cells in the same volume of blood.

Volume of cells in 100 ml. blood (PCV) = p ml
Volume of cells in 1000 ml. blood = 10 p ml
Number of cells in 1 cu.mm. blood (RC count) = r x 10^6
Number of cells in 1 ml blood = r x 10^6 X 10^3
Number of cells in 1000 ml. blood = r x 10^6 X 10^3 X 10^3
= r x 10^{12}

Volume of one cell $= \frac{10\ p}{r \times 10^{12}}$

= 10 p÷ r x 10^{-12} ml

10^{-12} ml = μ^3

Volume of one cell [MCV] = 10 p ÷ r μ^3

Mean Corpuscular Haemoglobin (MCH)

The haemoglobin content of one red cell is calculated by dividing the amount of haemoglobin in 100 ml of blood by the number of cells in the same volume of blood.

Amount of haemoglobin in 100 ml blood = h g
Number of cells in 100 ml. Blood = r x 10^6 x 10^3 x 10^2
Haemoglobin in one cell = h ÷ (r x 10^{11}) g.
= (10h ÷ r) x 10^{-12} g
= 10h ÷ r µµg (Pg).

Mean corpuscular Haemoglobin concentration (MCHC)

The concentration of haemoglobin in red cells is calculated by dividing the haemoglobin in 100 mi blood by the volume of cells in the same amount of blood.

Haemoglobin in 100 ml. blood (Hb) = h g.
Volume of red cells in 100 ml. Blood (PCV) = p ml.
Concentration of haemoglobin = h ÷ p x 100 %

Results: calculate the values and enter them in the computer.

Exercise

Explain the use of the haematological indices in the diagnosis of the diseases of blood. Discuss the reliability of each index.

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Discussion:

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Experiment B5a

MEASUREFMENT OF THE BLEEDING TIME AND CLOTTING TIME

Bleeding Time

The term "Bleeding Time" refers to the time taken for the cessation of bleeding from capillaries. The bleeding from capillaries is arrested by platelet plug and contraction of the pre-capillary sphincters and therefore it is a measure of platelet and capillary integrity.

Instruments:

Lancet and blotting sheet.

Method: (Duke Method)

Make a prick of the earlobe with the lancet after sterilizing and start the stopwatch. Blot the wound gently every 15 seconds. Observe the blotting sheet for the blood stain. The bleeding would have stopped when blood stain no longer appears on the blotting paper at which time the watch is stopped.

Note:

The puncture should be of standard width and depth in order to obtain reproducible results. Also this should not cause embarrassment to the patient by appearing as a large haematoma or wound. Other methods have been developed to overcome these problems.

Clotting time

This is the time taken for the blood to clot by formation of fibrin threads. This can be measured for venous blood by the test tube method and for the capillary blood by the capillary tube method.

Instruments:

Test tube and capillary tube

Test tube method: Demonstration

Collect 5 ml of blood by venipuncture and transfer to a test tube and start a stop watch. Tilt the tube gently every 30 seconds to see if the blood flows freely. The clotting has occurred when the blood remains as a solid and does not flow on tilting record the time taken.

Clotting time:…….

Keep the tube for some more time and observe a clear fluid appearing on top of the clot.

Exercise:
Name the fluid appearing above the clot: …….
Describe the mechanism of expression of this fluid:
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Capillary tube method: – to be done by everyone

Make a finger puncture and fill a capillary tube with the blood and start a stop watch. Tilt the capillary tube every 30 seconds. The blood does not flow when clotted. At this point, break a small piece of the tube gently and observe for small threads in the blood.

Precautions:

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	Name (male)	Bleeding Time (min)	Clotting Time (min)	Name (female)	Bleeding Time (min)	Clotting Time (min)
1						
2						
3						
4						
	Mean					
	SD					

Discussion:

- Describe the advantages and disadvantages of the two methods of measuring clotting time.

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Describe the importance of these tests in clinical practice.

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Experiment B5b

PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME AND FIBRINOGEN TIME

Instruments:

Test tubes, automated pipettes, steel rack, water bath, Prothrombin reagent, APTT reagent, Fibrinogen reagent.

1. Collect venous blood in anti-coagulated (citrate) tube. Avoid applying tourniquet during blood collection as it can affect the APTT.
2. To get platelet free plasma, centrifuge the sample at 4000 rpm for 5 minutes, leave for 5 minutes and again centrifuge at 4000 rpm for 5 minutes. So that the damage to RBCs can be minimized.
3. Maintain the centrifuged sample in a water bath at 37°C.
4. Keep the reagents to be added for each test also in the water bath for few minutes.

Prothrombin Time (PT)

The prothrombin time is a measure of the integrity of the extrinsic and final common pathways of the coagulation cascade. This consists of tissue factor and factors VII, II (prothrombin), V, X, and fibrinogen. The test is performed by adding calcium and thromboplastin, an activator of the extrinsic pathway. PT is extremely sensitive to the vitamin-K dependent clotting factors (factors II, VII, and X). Tissue factor (factor III) is a transmembrane protein that is widely expressed on cells of non-vascular origin, which activates factor VII during the initiation of the extrinsic coagulation pathway. A cascade mechanism results in fibrin production and clot formation. Factor VII (tested by the PT test only) has the shortest half-life and is the first factor to decrease with vitamin K deficiency.

1. Take 100 µl plasma in a test tube.
2. Add 200 µl of readily available Prothrombin reagent and start the stop watch at the same time.
3. Keep the tube at 37°C and frequently take out and observe for clot.
4. When you see the clot / the mixture is not moving, stop the stop watch and measure the time.

Note: This will be the PT of control. Follow the same procedure in patient's sample and measure the time taken to clot. Use the standard chart available with the reagent to find the PT of patient.

Activated Partial Thromboplastin Time (aPTT)

The aPTT test is used to measure and evaluate all the clotting factors of the intrinsic and common pathways of the clotting cascade by measuring the time (in seconds) it takes a clot to form after adding calcium and phospholipid emulsion to a plasma sample. The result is always compared to a control sample of normal blood.

Method:

1. Take 100 µl plasma in a test tube.
2. Add 100 µl of readily available aPTT reagent.
3. Add 100 µl $Cacl_2$ into that and start stop watch.
4. Keep the tube at 37°C and frequently take out and observe for clot.
5. When you see the clot / the mixture is not moving stop the stop watch and measure the time.

Interpretation:

The aPTT evaluates factors I (fibrinogen), II (prothrombin), V, VIII, IX, X, XI and XII.

When the aPTT test is performed in conjunction with prothrombin time (PT) test, which is used to evaluate the extrinsic and common pathways of the coagulation cascade, a further clarification of coagulation defects is possible. If, for example, both the PT and aPTT are prolonged, the defect is probably in the common clotting pathway, and a deficiency of factor I, II, V, or X is suggested. A normal PT with an abnormal aPTT means that the defect lies within the intrinsic pathway, and a deficiency of factor VIII, IX, X, or XIII is suggested. A normal aPTT with an abnormal PT means that the defect lies within the extrinsic pathway and suggests a possible factor VII deficiency

An aPTT that is grossly prolonged e.g. >120s is more likely to be due to a contact factor (XII) deficiency than to a deficiency of factor VIII or IX. Conversely an aPTT in the region of 70-80s is more in keeping with a diagnosis of severe haemophilia A (VIII) or B (IX) rather than a contact Factor Deficiency.

Fibrinogen Time (FT)

Fibrinogen time measures the rate of conversion of fibrinogen to fibrin in the presence of excess thrombin.

1. Prepare 1/10, 1/20, 1/30, 1/40 dilutions of the plasma.
2. Take 100 µl from each dilution and maintain in water bath at 37°C.
3. Add 100 µl of fibrinogen reagent to the first dilution and start the stop watch.
4. When you see the clot / the mixture is not moving stop the stop watch and measure the time.

5. Repeat the procedure for the other dilutions also.
6. Plot fibrinogen concentration against time taken to clot on a log-log graph.
7. Prepare 1/10 dilution for patient's plasma and measure the clotting time as indicated above.

8. Using the clotting time of patient extrapolate the fibrinogen concentration using standard curve.

Observations:

Prothrombin Time:……

Activated Partial Thromboplastin Time:…….

Fibrinogen Time:…….

Experiment B6

IDENTIFICATION OF LEUCOCYTES AND DIFFERENTIAL COUNT

The white cells are a group of different types of cells. It is very important to be able to identify the cells on a stained slide for counting the number of each type of cells. (It is presumed that the total white cell count is already done). The proportion of each type of cell in a stained blood smear is determined; the number of each type of cell is determined from the two values.

Instruments:

Slides, staining tray, Stop watch, and microscope with oil-immersion objective.

Reagents:

Leishman's stain: This is a mixture of eosin, methylene blue and some derivatives of methylene blue. The powder, containing all those in correct proportion, is dissolved in methyl alcohol (150 mg per 100ml). The stains are neutral salts of weak acids i.e. they are buffer salts. These salts can bind and give color to different substances only when they ionize and become active ions. This does not happen in alcohol solution. Eosin is said to be acidic stain because the staining is due to the anion: methylene blue is basic because the staining is due to cation.

Buffered water, pH 6.9: The buffered water is necessary to provide the medium for ionization of the dyes for staining.

Method:

Preparation of blood smear

Blood smear is made by first taking a drop of blood on a slide and spreading to form a thin film of blood. Take a drop of blood, from a finger prick. Near one end of a clean and dry glass slide. Take another slide which has sharp edges (this slide will be referred to as spreader) and place it on the middle of the first slide at an angle of 45. Draw the spreader, keeping an angle 45 to touch the drop of blood. The blood spreads along the edge of the spreader. Draw the spreader quickly towards the other end of the slide. (A good smear will be fairly uniform, neither too thick nor thin, and will have no uneven streaks or spots on it). Keep the slide until it dries completely.

Staining

Place the slide across two of the parallel supports on the staining tray so that the slide is horizontal. Add stain by a dropper just to cover the film (about 10-12 drops) and wait for two minutes. The alcohol fixes the cells firmly on the slide to prevent them from being washed away during the subsequent steps. No staining occurs during this phase.

Add about ten drops (equal to that of the stain) of buffered water by another dropper to the stain on the slide and mix it by blowing gently over the fluids until the solution has a uniform color. Wait for another seven minutes. Mix the solution

intermittently. (In a short while, thin film of sediment forms on the surface of the solution. The buffered water promotes ionization of the compounds in the solution and the stain is activated.

Pour off the fluid on the slide and wash the excess stain under a slow running tap. Stand the slide on its edge and let it dry completely.

Focusing under oil-immersion lens

Examine the appearance of the slide for the general quality of staining. A good smear is roughly rectangular with a rather dense and straight 'head end' and a thinner and convex 'tail end'. It is light purplish in color and translucent. Focus under the lowest power in the microscope and inspect the slide quickly for the distribution and appearance of the cells. Focus under the high power (40) and inspect the different areas of the smear. First distinguish between the numerous pink coloured red blood cells and the fewer large blue stained white blood cells. Then observe the distribution and appearance of the cells in different parts of the slide. At the head end the red cells are crowded and the white cells are poorly stained. At the extreme tail the cells are wide apart and white cells are distorted. The cells are stained well and seen clearly in the body of the smear near the tail end. Identify the best area (the body of the smear) for further study.

The detail structure of the individual cells can only be seen through the oil immersion objective (magnification 100). **Utmost care is needed when focusing under this objective** as the focal distance is less than 2mm. Lower the stage of the microscope further down and switch on (turn) the oil immersion objective to position while watching the stage and the slide to avoid any damage. If the objective lens is likely to touch the slide, lower the stage further down.

Place a drop of 'ceder wood oil' (immersion oil) on the blood smear and move the slide so that the oil (immersion oil) on the blood smear is directly under the objective. While watching the slide and the objective from the side and **NOT through the eye-piece of the microscope**, raise the stage until the oil touches the objective. Now look through the eye-piece and adjust the illumination (bright light is needed for clear vision). Looking through the eye-piece, raise the stage slowly until **suddenly** the cells come under focus. If clear image has not appeared within two or three turns of the knob, lower the stage and start focusing once again after ensuring that the illumination is adequate and that the slide contains cells (sometimes if the fixation was not properly done or if the slide was washed vigorously, the cells may be washed away. The slide may be upside down). The oil between the objective and the slide serves as a concave lens to increase magnification and reduces aberration of light and facilitates the entry of all light into the microscope.

Keep the cells under focus (by constant adjustment of the knob because the slightest alteration in the depth can affect the image) and move the slide about and study the structure of various types of cells and their size in relation to red cells. The red cells can be easily identified because they are pink non- nucleated discs found all over the field. You have to search for the white cells which will be seen as distinct cells with nucleus stained purple with clear or granulated cytoplasm. Remember that the cells are spheres and at any time the microscope will be focused only in one plane of the cell. Therefore, it will be necessary to adjust the focus up and down to see the cell in full.

Identification of leucocytes

Note the following points with regard to any leucocyte.

a. The size and shape of the nucleus.
b. Presence or absence of cytoplasmic granules.
c. When present- the size, number and staining reaction of the granules.

If the nucleus occupies only a small portion of the cell and if it is lobulated, the cell is a polymorpho-nuclear leucocyte. If there are three more clear lobes then the cell may be Neutrophil; if the lobes are clearly defined and arranged like spectacles then it is probably an eosinophil; but if the two lobes lie on top of each other because of the position of the cell, only one small lobe can be seen. The nucleus of the basophil is elongated and poorly divided into three lobes.

If the nucleus is not lobulated but spherical and fills almost all the cell then the cell is a lymphocyte. I the cell has a large kidney shaped nucleus, it is a monocyte; the nucleus of the monocyte can appear circular or even oval shaped depending on the orientation of the cell on the slide.

If cytoplasm is clear and light purplish in color, the cell is an agranulocyte. If there is only scanty cytoplasm then the cell is a lymphocyte. Lymphocytes can be found is sizes equal to red cells (small lymphocytes) or much larger than the red cells (large lymphocyte). If the cells are more than double the size of the red cell and lots of clear cytoplasm then the cell is a monocyte. If the cytoplasm is not clear but no definite granules can be seen and the nucleus is lobulated, the cell is a neutrophil. If the granules are bluish, large and discrete, then it is a basophil. If the granules are reddish and just distinguishable as discrete granules then it is eosinophil. The number of granules in an eosinophil is more than in a basophil.

The first and important part of the practical is to observe the different leucocyte under the microscope and gain experience in identifying them. If you see any cell then make sure that you identify it correctly by showing it to a teacher or a technician. When you come across a rare cell like monocyte, eosinophil or basophil, show it to others also. If you could not see one in your slide go around and see these cells in some others microscope. It is important that you become familiar with the appearance of all leukocytes before proceeding to the next part of the practical.

Exercise:
Draw each type of the white blood cell as you see in the microscope and label them.
Neutrophil: Eosinophil:

Basophil: Monocyte:

Lymphocyte:

Method of differential count:

100 small squares have been drown below. Move the slide so that the upper left corner of the selected field for counting is under focus. Look through the microscope and start moving the slide slowly towards right. Identify any leucocyte you come across and enter it in the squares: neutrophil –N, eosinophil—E, basophil—B, lymphocyte—L and monocyte – M.

Keep counting the cells until you come to the end of the field and then move the slide little towards you and go on counting while moving the slide towards left; when you come to the end of the field again move down and then towards right. This way you will avoid counting the same cell twice. Keep counting until the all the 100 squares are filled: you have recorded 100 cells. Now calculate the number of each type of cells from your record and the numbers will be the percentage of the leucocytes in blood.

Precautions:

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Results:

	Name (male)	N	L	E	M	B	Name (female)	N	L	E	M	B
1												
2												
3												

Discussion:

a. Describe the possible errors in the determination of the differential count.

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b. Describe the importance of total white cell count in interpreting the differential count.

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c. Describe the importance of **WBC/DC** in clinical practice.

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Experiment B7

BLOOD GROUPING

The blood is grouped according the antigen (agglutinogen) found on the membranes of red blood cells; antigens responsible for the ABO system and the Rh system are generally identified. They are identified by agglutination of red cells when reacted with known antibodies (agglutinins).

Instruments:

Blood grouping slide, hand lens.

Reagents:

Anti A (blue), anti B (yellow), anti D (colourless) and Normal saline

Method:

Take about 1 ml of normal saline in a small tube and add two or three drops of blood from a finger puncture and make a red cell suspension. Keep it for five to ten minutes during which time the blood might clot. Free the red cells from the clot (if present) by shaking the suspension

Take a drop of each anti- serum on the cavities of the blood grouping slide, as labeled. Add one drop of red cell suspension to each anti-serum and rock the slide gently in a circular motion for about ten minutes or till the agglutination is clear make sure that what appeared is agglutination and neither rouleaux formation nor clotting.

Note

Generally agglutination occurs with anti A and anti B fairly quickly and it is easy to observe. Agglutination with anti D is slow and does not show clearly. It may be necessary to wait for 20 minutes and use a magnifying glass.

Precautions:

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Observations

Name	A	B	AB	O	Rh		Name	A	B	AB	O	Rh	
					+ve	-ve						+ve	-ve
Total													
Percentage													

Discussion:

Explain the need for direct testing before blood transfusion.

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Describe the importance of grouping the blood of pregnant ladies.

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Describe the use of blood groups in medico-legal procedures.

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Nerves and Muscles

Experiment E1

HAND GRIP STRENGTH AND FATIGUE TIME

Muscle fibers contract when stimulated. Higher the number of motor units activated the higher the force generated by the muscle. When many fibers are depolarized simultaneously, the voltage differences in the overlying skin can be detected by surface electrodes. This recording of changes in skin voltage produced by underlying skeletal muscle depolarization is called Electromyography (EMG).

The strength of the hand grip can be measured by an appropriate transducer. Fatigue time is measured by doing forceful exercise continuously and measuring the time taken for the development of fatigue.

Instrument

Biopack system: Surface electrodes, Hand grip dynamometer

Method

Make the recording of the dominant arm of the subject first.

1. Attach three electrodes in the ventral aspect of the forearm as mentioned in the guide.
2. Clench the dynamometer with 5 kg force (software will display the screen to view your force level), hold for 2 seconds and then release**.** Wait for 2 seconds before beginning the next cycle
3. Repeat the step with increasing force (10, 15, 20…) in each cycle to compare the force generated at each clench.
4. Clench the hand dynamometer with maximum clench force and try to maintain it.
5. When the maximum clench force is decreased by 50% (fatigue) stop recording.
6. Repeat the above procedures in the other hand.

Observation:

Record the maximal clench strength of the dominant hand: ……

Record the fatigue time of the dominant hand: …….

Record the maximal clench strength of the other hand: ……

Record the fatigue time of the other hand: …….

Exercise

1. Describe the principle of EMG:

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2. List the factors that contribute to fatigue

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3. Comment on the differences between the results of both hands:

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Experiment E2

NERVE CONDUCTION STUDY

Conduction of impulses in the nerves is the basis of communication between the central nervous system and the sensors and effectors in the periphery. Study of this function is essential for diagnosing problems in these functions. Nerves can be stimulated by surface electrodes because the flow of electrons from cathode to anode sets up an electrical field which reduces the membrane potential of the nerve below the cathode to threshold level and hyper polarizes the nerve below the anode. The action potential spreads along the nerve and stimulates the muscle supplied by the nerve. Surface electrodes fixed over the muscles record the Electromyogram. The time taken for the EMG after the stimulation is measured **(latency**). Stronger stimuli will stimulate more axons and depolarization of more muscle fibers resulting in stronger response **(Amplitude)**. Low amplitude with stronger stimuli will indicate Partial block. If the nerve is stimulated at two different places, the difference in latency will be the time taken for the impulse to travel from the proximal point of stimulation and the distal point of the stimulation. Using this and the distance between the points, velocity of conduction can be calculated. These are widely used in diagnosis of peripheral nerve disorders.

When a motor nerve axon is stimulated while the cathode end faces the spinal cord, Impulse conducted proximally to the anterior horn cell through the spindle afferents will complete the reflex arc which also gives valuable information. This leads to depolarization of the muscle **(F wave)** after a longer latency.

Instrument: Nueropack EMG/EP measuring system

Demonstration

Subject sits comfortably in a chair. Recording electrode, ground electrode and surface stimulation electrodes are connected to the system. Patient's skin, where the recording electrode is attached, is cleaned with cotton moistened with alcohol. Skin is rubbed with Skinpure skin preparation gel to improve conduction and then wiped with dry gauze to remove any moisture. Earth is fixed between stimulating and recording electrodes.

Note: Cathode end of recording electrode should be fixed towards stimulation site and cathode end of stimulating electrode should face the muscle.

Motor nerve conduction (MNCS)

A. Median nerve

1. Recording electrode is placed over the abductor pollicis brevis (proximal).
2. Median nerve is stimulated between the tendons of flexor carpi radialis and palmaris longus at the wrist (distal) and medial to the palpable brachial artery at the elbow.
3. Stimulus strength is increased to obtain maximum combined muscle action potential.
4. Measure the distance between both stimulation sites to calculate the velocity.

B. Ulnar nerve

1. Recording electrode is placed over abductor digiti minimi.
2. Ulnar nerve is stimulated near the flexor carpi ulnaris tendon at the wrist and proximal to the sulcus nervi ulnaris (behind the medial epicondyle of the humerus).
3. Measure the distance between both stimulation sites to calculate the velocity.

Sensory nerve conduction (SNCS)

A. Median nerve

1. Ring electrodes are placed near second proximal interphalangeal joint and second distal interphalangeal joint.
2. Median nerve is stimulated at the same places as indicated for MNCS.
3. Measure the distance between both stimulation sites to calculate the velocity.

Ulnar nerve

1. Ring electrodes are placed near fifth proximal interphalangeal joint and fifth distal interphalangeal joint.
2. Ulnar nerve is stimulated near at the same places as indicated for MNCS.
3. Measure the distance between both stimulation sites to calculate the velocity.

Observations:

Median Nerve-

Latency for elbow motor stimulus: ……..

Latency for wrist motor stimulus: …….

Distance between both stimulatory cathode positions: ……

Latency for elbow sensory stimulus: ……..

Latency for wrist sensory stimulus: …….

Distance between both stimulating electrodes: ……..

Ulnar nerve-

Latency for elbow motor stimulus: ……..

Latency for wrist motor stimulus: …….

Distance between both stimulatory cathode positions: ……

Latency for elbow sensory stimulus: ……..

Latency for wrist sensory stimulus: …….

Distance between both stimulating electrodes: ……..

Calculation:
Calculate the conduction velocity of motor component of the ulnar nerve:

Calculate the conduction velocity of motor component of the median nerve:

Calculate the conduction velocity of sensory component of the ulnar nerve:

Calculate the conduction velocity of sensory component of the median nerve:

Exercise

1. Briefly describe the factors affecting the nerve conduction velocity.
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2. Comment on the observations:

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Respiratory System

INSTRUMENS USED IN RESPIRATORY EXPERIMENTS

Stethograph

The stethograph is a corrugated rubber tube, closed at both ends. The cavity of the tube is connected to a tambour by a noncompressible tube. Stretch on the corrugated rubber tube increases the volume which reduces the pressure in the system which pulls the drum of the tambour down. A pen on the tip of the pointer, connected to the tambour, writes on a moving drum which gives the pattern of the changes in the volume in the system. When the stethograph applied to the chest of a person, the chest movements can be recorded.

Spirometer:

The spirometer contains two concentric cylinders. The space between the two cylinders is filled with water in which an inverted cylinder (breathing chamber) moves up and down. There are two passages in the inner cylinder communication on the top with the breathing chamber and at the bottom with the inhalation and exhalation corrugated tubes; the breathing chamber can either be connected to the tubes (to the subject) or closed while the tubes open to atmosphere by rotating the union by a lever. The subject breathes from the breathing chamber when the union is at 'spirometer' and breathes from air when the union is turned to 'atmosphere'. The inverted cylinder is connected to a counter-weight, a piece of lead, by a string running over a system of pulleys. A pen, attached to the counter-weight, records movements of the inverted cylinder (changes in the volume of the breathing chamber) on a moving drum.

Douglas Bag:

This is a rubber or plastic bag used to collect expired air over a specific period.

Wet gas meter:

This is an instrument used to measure the volume of a gas at atmospheric pressure. The air pushed through the inlet rotates a wheel which is sealed by water. The wheel is connected to a system of dial which indicates the volume of the gas passed. Since the gas passes over water, the measured volume includes saturated water vapor.

Dry gas meter

This instrument is used to measure the volume of gas collected in Douglas bag. It consists of chambers formed by movable diaphragm. When the gas is allowed to go through the inlet, it changes the relative pressure in the chambers & they alternatively get filled. So the diaphragm moves like bellow. Levers connected to diaphragm rotate a crank shaft, which drives a counter mechanism to transform the volume of gas pass through in cubic meter on the outside dial.

The reading can be taken up to three decimal points. Initial reading of the gas meter has to be taken before allowing the gas to pass through the meter. Next reading will be taken after the gas has been passed through the meter. Difference between both readings will give the volume of the gas that has passed through the gas meter, in cubic meters. Multiplying this value by 1000 will give the volume in liters.

Experiment R1

GRAPHIC RECORDING OF RESPIRATION

The circumference of the chest increases during inspiration and it decreases during expiration. This principle is used to record and study the qualitative aspects of respiratory movements.

Instrument:

Stethograph

Method:

Check the system for leaks first; adjust the recording pen to write on the kymograph drum; blow through the system to increase the pressure slightly so that the pen rises about 2 cm and clamp the opening; rotate the drum to draw a line of about 2 cm; and leave the system undisturbed for 2 minutes. If there is any leak, the pen will drop down.

Apply the stethograph to the chest of the subject snugly and adjust the pressure in the system to give a deflection of about 2.5 cm with each normal breath. Record the chest movements while the subject performs the following procedures.

1. The subject breathes quietly at rest.
2. The subject takes a deep breath and holds the breath until the breaking point is reached. Keep recording until the breathing comes back normal pattern.
3. The subject breaths pure oxygen and holds breath at the end of normal inspiration until breaking point is reached. Keep recording until respiration comes back normal pattern.
4. The subject breathes as fast and deep as possible. The subject relaxes when it becomes impossible to breath any more. Keep recording until the respiration comes back to normal.
5. Allow the subject to relax and connect one-meter long tube to the mouth piece with the nose closed. Remove the tube when a steady state of breathing is reached and record until the breathing comes back to normal pattern after removing the tube.
6. The subject coughs and sneezes violently.

Observations

Stick the photocopy of the tracing

Exercise

1. Quiet Breathing:

Calculate the respiratory rate: ----------

Comment on the pattern of inspiratory and expiratory tracing: ----------
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2. Breath Holding after deep inspiration:

Duration of breath holding: ----------
Rate of Respiration after 1 minute: ----------
Rate of Respiration after 2 minutes: ----------
Rate of Respiration after 3 minutes: ----------
Comment on the width of tracing at the breaking point and thereafter: ----------
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3. Breath Holding after breathing Oxygen:

Duration of breath holding: ----------
Rate of Respiration after 1 minute: ----------
Rate of Respiration after 2 minutes: ----------
Rate of Respiration after 3 minutes: ----------
Comment on the amplitude of tracing at the breaking point and thereafter: ----------
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4. Hyperventilation:

Calculate the respiratory every minute after stopping the hyperventilation.
Rate of Respiration after 1 minute: ----------
Rate of Respiration after 2 minutes: ----------
Rate of Respiration after 3 minutes: ----------
Comment on the change in amplitude of the tracing: ----------
---

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5. Breathing through a tube:

Rate of Respiration after 1 minute: ----------
Rate of Respiration after 2 minutes: ----------
Rate of Respiration after 3 minutes: ----------
Rate of Respiration after 4 minutes: ----------
Rate of Respiration after 5 minutes: ----------
Rate of Respiration after 6 minutes: ----------

---

6. Cough and sneeze:

Describe the phases of cough based on the tracing: ----------
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Describe the phases of Sneezing based on the tracing: ----------
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Discuss the observations in the above procedures:

Experiment R2

MEASUREMENT OF LUNG VOLUMES

Measuring the volume of the lung is difficult because it is an elastic organ which contains air. The amount of air in the lung varies depending on the state of respiration. Therefore the volume should be measured under defined conditions. Another reason for the difficulty in measuring the volume is its irregular internal structure. The volume can be measured either by dilution of non-absorbable gases or by measuring the air expelled from the lung. The first method cannot be employed in our laboratory. The second method is spirometry. Since all air in the lung cannot be expelled out, this method is inappropriate to measure the absolute volume of the lung. Changes in the lung volume, however, can be measured which is very useful in assessing lung function. Lung volumes can also be predicated from measurements of height and age fairly accurately.

Exercise:

Define:

a. Tidal volume

……
……

Expiratory reserve volume

……
……

Inspiratory reserve volume

……
……

Vital capacity

……
……

Forced vital capacity

……
……
……

Force expiratory volume in the first second

……
……
……

Instrument:

Spirometer: electronic for demonstration, mechanical for measurements by the students.

Method:

The subject sits comfortably in a chair, facing away from the spirometer. Turn the union of the tubes to the 'spirometer' position and flush the breathing chamber with fresh air by raising and lowering the inverted drum. Raise the inverted cylinder half way up and turn the outlet to 'air' which closes the inner tube and allows the subject to breathe from atmosphere. The subject applies the mouthpiece and breathes through the corrugated tube. The nose of the subject is close by a nose clip. Wait until the subject gets used to the apparatus. **Instruct the subject about the procedures to be followed before each measurement.** Make sure that the measurements are completed soon or disconnect the subject from the spirometer and collect air in the drum.

Tidal volume:

Switch on the Kymograph of the spirometer at low speed and turn the union 'spirometer' which makes the subject breathe from and into the spirometer.

Expiratory reserve volume:

Ask the subject to expel all possible air out of the lungs and breathe as usual: make two recordings.

Inspiratory reserve volume:

Ask the subject to inhale as much air as possible and breathe as usual make two recordings.

Vial capacity:

Ask the subject to inhale as much air as possible and expel as much as possible and then breathe as usual: make two recordings.

Forced vital capacity:

Ask the subject to inhale as much air as possible and increase the speed of the recording drum (25 mm/sec). Ask the subject to expel air as much and as rapidly as possible.

Calculate the forced expiratory air volume in the first second and express it as a percentage of the total air expelled (FEV_1).

Method of prediction of lung volumes:

Lung volume depends on the build of a person. The lung volume therefore, may be expected to be related to the height and weight of a person. Formula give below are used to predict the volumes.

VC = 0.064 X [height (cm)] – 0.031 X (age) – 5.335 Male
= 0.052 X [height (cm)] – 0.018 X (age) – 4.36 Female

Total lung capacity (TLC)

TLC = 0.094 X [height (cm)] – 0.015 X (age) – 9.617 Male
= 0.097 X [height (cm)] – 0.008 X (age) – 7.49 Female

Observations

Name (male)	Predicted		TV (l)	IRV (l)	ERV (l)	VC (l)
	VC (l)	TLC (l)				
Mean						
SD ±						

Name (female)	Predicted		TV (l)	IRV (l)	ERV (l)	VC (l)
	VC (l)	TLC (l)				
Mean						
SD ±						

Exercise:

Explain the need for changing the air in the breathing chamber of the spirometer frequently.

Discuss the observations:

Experiment R3

CHEMICAL CONTROL OF RESPIRATION

The chemoreceptors sense the partial pressures of oxygen, carbon dioxide and acidity in arterial blood. When these are altered, they send impulses to the respiratory center to bring about compensatory adjustments in respiration. The effects of changes in the gas concentration can be demonstrated in two ways; one is by altering the partial pressure of oxygen, carbon dioxide or both in the inspiratory gas and observing the changes in breathing; the other method is studying the changes in breath holding time after altering the gas concentrations.

Instruments:

Spirometer
Oxygen analyzer
Carbon dioxide analyzer

Method:

Collection and analysis of alveolar air

Take a normal breath and expire through a narrow tube which is opened on both ends. The dead space air comes first and escapes through the other end. The alveolar air comes last and remains in the tube. Connect the tube to the inlet of the carbon dioxide analyzer. The pump in the carbon dioxide analyzer draws the alveolar air and pushes through the analyzer. The out let is connected to a drying column containing silica gel which leads to the oxygen analyzer. Read the maximum value from the carbon dioxide analyzer and the minimum value form the oxygen analyzer.

Changes in Breathing

Measure the resting alveolar gas concentrations of the subject. Also record the pulse rate every minute during each of the following procedures which will indicate the effects of chemoreceptor stimulation on heart.

Effects of Hypoxia

Do not perform this procedure unless a lecturer or a demonstrator supervises because it is dangerous.

Collect fresh air in the breathing chamber of the spirometer. Connect the tubing to pass the expiratory gas through the soda lime canister to the breathing chamber. Record the resting pulse rate and turn the handle and allow the subject to breathe from the spirometer and record the movement of the bell. At the same time ask the subject to keep writing a sentence again and again. Observe the skin for changes in color. Keep recording the pulse rate every minute. Stop the procedure when you see changes in the writing. Measure the alveolar gas concentration immediately, before breathing atmospheric air. Ask the subject for any unusual feeling.

Stick the photocopy of the tracing

Exercise:

a. Alveolar gas concentrations:……..
b. Tabulate the respiratory rate and tidal volume every minute and explain the changes.

Time (minutes)	Pulse rate	Respiratory rate	Tidal volume
0 (Resting)			
1			
2			
3			
4			
5			
6			
7			
8			
9			

c. Explain the changes in pulse rate, respiration, skin color, handwriting and unusual feeling experienced.

---

d. Explain the danger in this procedure.

Effects of hypercapnoea

Fill the breathing chamber with pure oxygen and remove the soda lime canister. Make the same recording. (No need to keep writing). Stop recording when the subject becomes restless or feels very uncomfortable. Measure the alveolar gas concentration before breathing from atmospheric air.

Stick the photocopy of the tracing

Exercise:

Time (minutes)	Pulse rate	Respiratory rate	Tidal volume
0 (Resting)			
1			
2			
3			
4			
5			

a. Alveolar gas concentrations:……
b. Explain the changes in respiration and pulse rate.

---

c. Explain why this is a safe procedure.

Effects of Asphyxia

Collect fresh air in the breathing chamber and allow the subject breathe and make the same recordings.

Stick the photocopy of the tracing

Exercise:

Alveolar gas concentrations:……..

Time (minutes)	Pulse rate	Respiratory rate	Tidal volume
0 (Resting)			
1			
2			
3			
4			
5			

Explain the changes observed. Compare the changes with the earlier recordings.

Changes in breath holding time

Method:

Analyze the alveolar gas concentrations after each of the following procedures. Ask the subject to hold breath as long as possible after repeating each of the following procedures and when the breaking points are reached to expel the air in the lungs through the tube to analyze the alveolar gas again. This is to obtain the gas concentrations at the beginning and at the end of the breath holding period. The subject should signal at the start and end of the breath holding in order to start and stop the stopwatch.

a. Breathing pure oxygen for one minute (end of deep inspiration).
   Breath holding time:…….
   Alveolar Oxygen:……
   Alveolar Carbon Dioxide:…….....

b. Normal inspiration.
   Breath holding time:…….
   Alveolar Oxygen:……
   Alveolar Carbon Dioxide:…….....

c. Deep inspiration.
   Breath holding time:…….
   Alveolar Oxygen:……
   Alveolar Carbon Dioxide:…….....

d. Hyperventilation as long as possible.
   Breath holding time:…….
   Alveolar Oxygen:……
   Alveolar Carbon Dioxide:…….....

Exercise:

Correlate the breath holding time with alveolar gas concentration and explain the inferences.

Experiment R4

EFFECT OF EXERCISE ON VENTILATION

There is increase in the rate and depth of respiration when a person engages in muscular exercise. The aim of this practical is to correlate the changes in respiration with the degree of exercise and to study the changes in alveolar gas concentrations. The concentrations of oxygen and carbon dioxide in the alveolar air reflect the partial pressures of the gases in the arterial blood.

Instruments:

Cycle ergo meter, Douglas bag, Double flutter valve, Gas meter, Oxygen analyzer, and Carbon dioxide analyzer

Procedure:

First measure the resting parameters. Seat the subject comfortably in a chair. Connect the mouth peace to the double flutter valve and connect the valve to a Douglas bag through a corrugated rubber tube. Let the subject fix the mouth piece and allow the expired gas to escape to atmosphere through the tap on the Douglas bag. Collect the expired air for five minutes by turning the tap of the Douglas bag when the subject is relaxed. Count the respiration over the period. Collect and analyze the alveolar gas

Seat the subject on the cycle ergometer with the Douglas Bag connected and ask the subject to pedal at a rate of 15 Km/hr, against a resistance of 2 kg. After 4 minutes, when the subject has reached a steady state, start collection of expired air and count the respiratory rate (the subject continues to pedal). After 2 minutes disconnect the Douglas bag and collect alveolar air and analyze the gas concentrations. Allow the subject to rest.

Repeat the same measurements while the subject pedals at 30 Km/hr.

Calculate the rate of respiration, tidal volume and minute ventilation.

Observation

	Subject at Rest						Subject during exercise at											
							20 Km/hour						30 Km/hour					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Minute Ventilation																		
Respiratory Rate																		
Tidal Volume																		
% of CO_2 in alveolar air																		
% of O_2 in alveolar air																		

Subject Name

1. ……
2. **.....**
3. …….
4. …….
5. …….
6. ……..

Exercise:

a. Explain the changes observed in the parameters.

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b. Describe the factors that affect respiration in exercise.

Experiment R5

EVALUATION OF THE RSPIRATORY SYSTEM

The aim of the practical is to illustrate the relevance of learning respiratory physiology to clinical situation.

Functions of the respiratory system can be assessed either by clinical examination and or by investigations. All the results are put together to identify diseases and to decide on the management. All these are repeated in order to monitor the progress of the patient.

Clinical evaluation

First step in clinical evaluation is to obtain a detail history and analysis of the symptoms; this is followed by physical examination and analysis of the sings.

History:

Recording the history of the illness starts with inquiring the age, sex, occupation and the address of the patient. This, while helping to open the discussion, provides valuable information.

How does the above information help?

Ask the patient about the presenting illness.

What complaints are relevant to respiratory system? What are the physiological bases of the above symptoms?

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Second step is to perform physical examination.

Inspection:

Observe the face skin mucus membrane for signs.

Surface mark the lungs. Determine the respiratory rate. Observe for any deformity of the chest. Observe for any difference in movement between both sides of the chest on deep respiration.

What observations are relevant to respiratory system?

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……

Palpation:

Locate the trachea and the apex of the heart and determine the position of the mediastinum.

What changes in the lung shift the position of the mediastinum?

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……

Fix the fingertips on the sides of the chest of the patient allowing the tips of the thumbs to meet at the midline. Ask the patient to take a deep breath and observe the movement of the thumbs to confirm the earlier observation regarding the movement of the chest. Perform this test for upper, middle and lower portions of the anterior and posterior chest.

What defects in the respiratory system alter the movement of the chest?

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Place the most sensitive portion of our hand on the chest and ask the patient to say 'ninety nine' or a similar word which has a nasal sound and feel the "vocal fremitus". Vocal fremitus is the vibration of the sound transmitted through the lungs and the chest wall.

What changes in the respiratory system increase or decrease the vocal fremitus?

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……..

Percussion:

The middle finger of the left hand is placed firmly on the part to be percussed. The back of the middle phalanx is then struck with the tip of the middle finger of the right hand; the force for strike should be obtained only from the movement of the wrist and inter phalanigal joints. The quality of the sound depends on the nature of the structure percussed. Solid structures produce dull sound and structure containing air produce resonant sound. Listen and identify the normal resonance.

How does percussion help in surface marking? What conditions lead to change in the resonance?

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……

Auscultation:

Auscultation is listening to the sounds produced in the chest with a stethoscope. Three observations can be made: character of breath sounds, vocal resonance, and abnormal sounds.

There are two types of breath sounds vesicular breath sounds (sounds produced by movement of air in and out of normal alveoli); and bronchial breath sounds (sounds produced by passage of air through trachea and large bronchi). Listen to the vesicular breath sound over different part of chest and observe the nature of the sounds and the differences between inspiration and expiration. Listen to the bronchial breath sound over the trachea and observe the same characteristics.

When could you hear bronchial breath sounds over the chest?

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……

Vocal resonance is produced in the same way as vocal fremitus. The only difference is auscultation instead of palpation.

Several added sounds can be heard in diseased lungs. Common sound heard is crepitation which is crackling or bubbling sound. Another sound is ronchi produced by air passing through partially obstructed air ways.

Investigation

Many investigations could be done and a common investigation is X – ray examination

Function tests:

Lung function tests are carried out in patients for physiological diagnosis in the following circumstances:

1. To give an objective assessment of patient's disability.
2. To follow the progress of a decease and the effect of treatment.
3 To differentiate the possible causes of dyspnoea.
4. To assess patients before anesthesia especially for thoracic surgery.

<u>Perform the following tests and record the parameters of the class.</u>

a. Maximum Breathing Capacity [MBC]:
Use douglas bag and dry gas meter . After setting up, collect the expired air with maximum possible dept and rate for 30 seconds. Measure the volume and calculate liters/minute.

b. Vital Capacity:
Use spirometer as described before.

c. FEV, FEV_1%-
Use spirometer to measure and calculate as done before.

d. Peak Expiratory Flow Rate:
Use peak expiratory flow meter. Ask the subject to take a deep inspiration and expel as fast possible through the peak flow meter. Hold the meter horizontally; bring the rider to zero before each reading. Record three readings and take the maximum reading.

<u>Observation</u>

Name (male)	MBC l/min	VC (l)	FEV (l)	FEV_1 %	PEF l/min	Name (female)	MBC (l/min)	VC (l)	FEV (l)	FEV_1 %	PEF l/min
mean											
SD ±											

Exercise

Explain their significance of the Following.

a. Vital Capacity.

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b. Forced Vital capacity.

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c. Peak Expiratory Flow Rate.

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d. Maximum Breathing Capacity.

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e. Discuss the use of other Lung Function Test such as blood gas analysis.

Cardiovascular system

INSTRUMENTS USED IN CARDIOVASCULAR PRACTICALS.

Stethoscope

It is a device to hear sounds comfortably without having to place the ear on the source of a mild sound. The tube is made of material that does not absorb sound energy. The receiver is placed on the source of a sound to be heard. The bell is used to pick high pitched sounds and the diaphragm is used to hear lower frequency sounds.

Sphygmomanometer

The sphygmomanometer consists of three parts: a compression cuff, a pressure source and a measuring device.

Compression cuff

The compression cuff consists of inflatable rubber bladder within an inelastic covering. Usually the cuff is applied over the arm. Since this has to press the artery through the skin and muscles, the energy spent to compress these structures should be comparable among different individuals. The standard size of the inflatable rubber bladder used for an average adult is 23 cm long and 13 cm wide. The width is the crucial factor and a recognized rule is that the width of the bladder be 20% greater than the diameter of the limb on which it is used. Recommended widths of compression cuffs for children of different ages are 2.5 cm for infants, 6 cm for 1-4 years and 9 cm for 5-8 years.

The covering of the inflatable bladder is made of inelastic material and provides uniform pressure over its full width. The cloth of the bandage type covering extends beyond the bladder sufficiently to encircle the arm several times. Modern cuffs are equipped with fasteners to make the application easy. Two mating surfaces of a special interlocking fabric (Velcro) lock the cuff firmly in place. The accuracy of blood pressure measured by the both types of the cuff is the same.

Pressure source

The pressure source consists of a rubber hand bulb and a pressure control valve. It is designed to be held in the palm, covered by the last three fingers while the thumb and the index finger are used to regulate the valve. The device is connected to the inflatable bladder by a rubber tube.

Measuring device

Three types of measuring devices are used: mercury – gravity manometer, aneroid manometer or electronic transducer. The mercury manometer consists of a straight glass tube in assembly with a reservoir containing mercury. The reservoir is connected to the inflatable bladder by a rubber tube. The pressure in the cuff is equal to the vertical height of the mercury column in the glass tube.

The aneroid barometer consists of a metal bellows, the inside of which is connected to the compression cuff. Variations of pressure within the system cause the

bellows to expand and collapse. Movement of the bellows rotates a gear that turns a needle, pivoted on bearings across a calibrated dial.

Advantages and disadvantages of the two types of sphygmomanometer

Mercury – gravity	**Aneroid**
a. Great and permanent accuracy and no need for re-calibration	a. Requires frequent calibration
b. Relatively bulky	b. Small and portable
c. Must be in vertical position while in use	c. Can be used in any convenient position
d. Breakable glass parts but easily replaceable	d. Must be factory repaired if becomes defective.

Cycle ergo-meter

It is a stationary cycle conveniently used for the performance of measured amount of work in the laboratory where scientific measurements could be made while performing the exercise. The seat, handle and the pedal resemble ordinary bicycle but wheel is lifted from the round. Varying amount of resistance can be applied through adjustable belt or suitable brake system. The work therefore can be altered in two ways: speed and load.

Treadmill

It is a moving platform on which the subject walk or run at predetermined speeds of the platform so that the subject will be in the same position for easy monitoring of body parameters.

Metronome

It is a time device which could be set to produce loud ticking sounds at the preferred time interval.

Plethysmograph

It is a rigid structure, which covers an organ. When connected to appropriate sensor, changes in the volume within the instrument can be recorded and measured.

Volume recorder

It is an instrument to record volume change. It has a water bath in which an inverted drum float, the space of the inverted drum is connected to the area where the volume change is to be recorded. Displacement of air between the drum and the measuring area will represent change in volume.

Experiment C1

ELECTROCARDIOGRAM

Electrical activities in excitable tissues results in fluctuation in the charges between different parts of the tissues. These charges cause feeble electrical fields in the body. When many cells generate electrical fields at the same time in an organized manner, electrodes placed on the surface of the body will be to pick up the net changes in the electrical field. These changes when amplified and fed to appropriate recorder, a meaningful picture is obtained. In this way activities of the skeletal muscles could be recorded as electromyogram and that of the heart as electrocardiogram. Activities in the nerves produce feeble electrical fields and very sensitive instrument are needed to record their activities.

The electrodes for recording electrocardiogram are of two types. One set is fitted to the limbs which connect the trunk to the machine at the points of Eintovan's triangle. These electrodes record the changes in the electrical field in the sagittal plane and help to provide zero potential for the unipolar leads. The other is a chest electrode, which can be moved along the chest and helps to record the changes in the cross sectional plane.

Instrument:

Electrocardiograph

Demonstration:

Fitting the electrodes

The subject lies comfortably in a bed. The subject is instructed to lie still and to make no muscular movements. The limb electrodes are wetted by saline for better contact with skin and secured in place by a rubber strap at the fore arms and legs above muscle belly. If the skin is hairy the place may have to be shaved to ensure god contact. The chest electrode is secured to the appropriate place by creating vacuum around the electrode. When all leads are connected to the electrocardiograph according to the color code, the necessary connections can be obtained by positioning the appropriate knob.

Recording

The standard setting of the electrocardiograph is to give 10 mm deflection for 1 mV and the paper movement of 25 mm per second. The machine is switched on and about five beats are recorded with the knob at lead I. Then the knob is turned to lead II. Lead III, aVR, ave & aVL. The knob is then turned to V which stands for chest leads, and the chest electrode is placed at the appropriate places on the chest to record V1 to V6 the tracing is removed and the leads are disconnected.

Observation:

Stick the ECG tracing

Exercise:

a. Study the tracing obtained for the following:
   1. Heart rate
   ……
   ……
   2. Rhythm:
   ……
   ……
   3. Pattern of P wave, QRS complex, and T wave.
   ……
   ……
   4. PR interval
   ……
   ……

b. Correlate the events in cardiac excitation to the waves in ECG

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c. Outline the use of ECG in clinical practice.

Experiment C 2

MEASUREMENT OF BLOOD PRESSURE

Blood pressure is the lateral pressure exerted by the blood on the artery wall at the level of the heart. In experimental animals this can be measured by connecting a manometer directly to the artery. The blood pressure of man is measured by an indirect method; a measurable pressure is applied over the artery; the pressure required to compress the artery is equal to the pressure. Measuring blood pressure is an accurate and simple method of assessing the state of the circulatory system.

The main aim of this practical is to gain skill in measuring blood pressure accurately

Instruments:

Sphygmomanometer and stethoscope

Method:

Make sure that the subject (or patient) is comfortably seated or in bed and in a relaxed condition. Remove cloths from the arm and apply the cuff.

Application of the cuff**:**

Blood pressure is generally recorded in the arm with the patient in sitting or recumbent position. The artery over which the pressure is to be measured should be at the level of the heart. The deflated compression cuff is applied evenly and snugly but without constriction around the arm. The lower edge of the cuff should be about 2.5 cm above the elbow joint. Make sure that the middle of the rubber bag is over the brachial artery, in the medical aspect of the arm.

Palpatory method of measurement:

This method is based on the fact that the pulse wave is not conducted through when the brachial artery is occluded. This happens when the external pressure (in the cuff) is more than the maximum pressure in the artery.

First, locate and palpate the redial or brachial pulse. While feeling the pulse by one hand, inflate the cuff by the other hand. Continue to increase the pressure for about further 30 mm Hg after the disappearance of the pulsation. Deflate the cuff slowly (about 2 or 3 mm Hg per second) until the pulse is felt again. The pressure at this moment is equal to the systolic blood pressure.

Auscultatory method of measurement:

This method is based on sounds produced at the lower end of the partially compressed artery due to turbulence. If the pressure in the cuff exceeds systolic pressure the artery is closed and there will be no sound. If the pressure in the cuff is below the diastolic pressure also there will be no sound because the artery is fully opened and there is laminar flow. When the cuff pressure is between systolic and diastolic pressures, the

artery closes when the pressure in the artery goes below the cuff pressure and the blood flows through when the pressure in the artery goes above the cuff pressure. The intermittent flow produces turbulence at the end of the cuff, which produces a sound (Korotkoff sound).

Locate the brachial artery in the cubital fossa by feeling for the pulse. Inflate the cuff to a pressure above the systolic pressure, measured by palpatory method. Place the stethoscope gently on the brachial artery and reduce the pressure slowly. The pressure at which the Korotkoff sound appears is the systolic pressure. Go on reducing the pressure while listening. Suddenly at one point the sound becomes muffled and when the pressure is lower lowered further the sound disappears.

There is a controversy as to the measurement of diastolic pressure. Some doctors consider the muffing point as the diastolic pressure while others consider the point of disappearance as the diastolic pressure. In this lab, the point of disappearance will be considered as the diastolic pressure. Repeat the measurements three times and record the lowest value. If you could not get the reading within two or three minutes, release the cuff pressure and start all over again after few minutes.

PHASES OF THE KOROTKOFF SOUNDS

Phase 1	beginning of a sharp “Thud” ---	Systolic pressure
Phase 2	a blowing or swishing sound	
Phase 3	a softer thud than phase 1	
Phase 4	a soft blowing sound [muffling] - -	First diastolic pressure
Phase 5	silence ----------	Second diastolic pressure

Observations

Name (male)	Palpatory	Auscaltatory		Name female)	Palpatory	Auscaltatory	
	SBP	SBP	DBP		SBP	SBP	DBP
Mean							
SD							

Exercise:

a. Explain the effect of a cuff, which is too narrow or to wide, on the measurement of blood pressure.

---

b. Explain the difference observed in the systolic pressure between palpatory and auscultatory methods.

---

c. Explain the factors that cause difference in the blood pressure in the same person at different times and among different person and the need for repeated measurements of blood pressure.

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d. Explain the need for quick measurements.

Experiment C 3

EFFECTS OF POSTURE AND INTRATHORACIC PRESSURE (TEST FOR PHYSICAL FITNESS I)

Physical fitness is the state of readiness and ability to perform physical work. The fitness cannot be assessed by any single test. Tests for physical fitness are classified into three main groups: muscular performance, organic function and combination of organic function and muscular performance. The first category of tests are simple and straight forward, two test for organic function will be carried out in this class. A test with a combination of muscular work and organic function will be carried out at a subsequent class. These tests are based on assessing the cardiovascular response to different types of stress which are mediated by autonomic nervous system.

Effects of Posture

Crampton's index for vasomotor tone

This test is based on the change in pulse rate and systolic blood pressure when posture is changed from reclining to standing.

Instruments

Sphygmomanometer, Stethoscope and a bed

Method

Allow the subject to relax on a bed (reclining) for at least five minutes and measure the systolic blood pressure and the pulse rate. Repeat the reading until the subsequent results agree. Then let the subject stand upright with minimal muscular effort. (Ideally the bed should be tilted by 90°). After two minutes of standing measure the blood pressure and the pulse rate.

Calculation

1. Calculate the changes (standing – lying pulse rate and systolic pressure)
2. Determine the index of fitness from Crampton's index for fitness.
3. Explain the physiological basis of the changes in heart rate and blood pressure.

CARMPTON'S INDEX FOR PHYSICAL FITNESS

Change in HR	Chang in systolic pressure										
	+ 10	+ 08	+06	+ 04	+ 02	00	- 02	- 04	-06	- 08	- 10
0 -4	100	95	90	85	80	75	70	65	60	55	50
05 – 08	95	90	85	80	75	70	65	60	55	50	45
09 – 12	90	85	80	75	70	65	60	55	50	45	40
13 – 16	85	80	75	70	65	60	55	50	45	40	35
17 – 20	80	75	70	65	60	55	50	45	40	35	30
21 – 24	75	70	65	60	55	50	45	40	35	30	25
25 – 28	70	65	60	55	50	45	40	35	30	25	20
29 – 32	65	60	55	50	45	40	35	30	25	20	15
33 – 36	60	55	50	45	40	35	30	25	20	15	10
37 – 40	55	50	45	40	35	30	25	20	15	10	05
41 - 44	50	45	40	35	30	25	20	15	10	05	00

(Good health – 60 -100%; some disturbance – 50% and severe physical disability> 10%)

Valsalva Manoeuvre- Increased Intra-thoracic Pressure

This manoeuvre depends on the effects on intra thoracic pressure (and intra-abdominal pressure) changes on venous return and consequently of pulse rate and blood pressure.

Note: Those who are known to have cardiovascular problems are requested not to do the Valsalva manoeuvre.

Methods:

After obtaining steady resting readings of pulse rate and blood pressure (systolic and diastolic) ask the subject to take a deep breath, and hold the breath for 30 seconds. Count the pulse / heart rate every 10 seconds for one minute (during and after breath holding) and the blood pressure half way through and after beginning of breathing. This is a control manoeuvre.

Ask the subject to perform Valsalva manoeuvre and repeat the measurements in the same manner. The subject takes a deep breath and closes the glottis; then the subject increases the intra-thoracic pressure by forceful contraction of all chest and abdominal muscles (straining) for 30 – 45 seconds. Carry out the measurements as before and continue until the readings come back to resting values. Ask the subject to describe any unusual sensation or feeling experienced during the manoeuvre.

Demonstration

The biopack system is used to demonstrate continuous changes in pulse / heart rate in both of the above experiments on one subject.

Name (male)	**Effects of Posture**									**Valsalva Manoeuvre**											
	Standing			Lying			Difference			Control (HR/10 sec)						Manoeuvre (HR/10 sec)					
	Pulse	SBP	DBP	Pulse	SP	DBP	Pulse	SBP	Fitness index %	Resting	10	20	30	40	50	Resting	10	20	30	40	50

Name (female)	**Effects of Posture**									**Valsalva Manoeuvre**											
	Standing			Lying			Difference			Control (HR/10 sec)						Manoeuvre (HR/10 sec)					
	Pulse	SBP	DBP	Pulse	SP	DBP	Pulse	SBP	Fitness index %	Resting	10	20	30	40	50	Resting	10	20	30	40	50

Exercise

a. Explain the physiological basis of the changes observed.

---

b. Explain the physiological basis of any unusual sensation.

Experiment C 4

ISCHAEMIC PAIN

When the blood supply of an active organ is inadequate, there occurs a crippling pain which is distinct from muscular fatigue and cramp. Since this is related to ischaemia, it is called ischemic pain. Ischaemia can be absolute when a blood vessel is completely blocked; it can be relative when the blood supply is less than required, generally, as a result of partial obstruction of vessels. The pain of angina pectoris, myocardial infarction pulmonary infarction and intermittent claudication of calf muscle are caused by ischaemia

The aim of this practical is to study some aspects of the causation of this pain and to give an experience of the nature of this pain to students. The efficacy of varying combinations of exercise and arterial occlusion in producing ischaemic pain is assessed.

Principle:

Varying combinations of exercise and arterial occlusion are carried out in producing ischaemic pain, in order to determine the role of each of these factors in the production of the pain.

Apparatus:

a. A wooden rod, stop watch and sphygmomanometer cuff.

Procedure:

Students will work in pairs: One student will make the observations on the other. The subject must be seated comfortably, with his right arm resting on a table. The following procedures are to be carried out on the same arm in the given order, allowing the arm to recover fully after each procedure before the next is begun.

A. Exercise the right forearm of the subject for four minutes, by gripping the wooden rod in the manner mentioned above.

B. Occlude the circulation through the right arm for four minutes, without any exercise. Describe the sensations in the arm during occlusion and on release of pressure.

C. Now combine A and B by performing the exercise after occluding the circulation in the arm. Note the time of onset of pain. Continue the exercises till the pain is unbearable, and note the time again. Then release pressure. Note the quality of pain and the time taken for the pain to disappear after the pressure is released.

D. Repeat C but reduce the rate of exercise to once in two second.

E. Repeat C but increase the rate of exercise to twice every second.

F. Repeat C. When the pain is quite marked, stop the exercise but maintain the pressure in the cuff for another minute and then release it.

Individual observations:

Procedure	Onset of pain (sec)	Onset of Un-bearable (sec)	Disappearance of pain (from release of pressure)	Other Sensations and observations
A				
B				
C				
D				
E				
F				

Class results:

Name	A			B			C			D			E			F		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
[X- onset of pain, Y- onset of unbearable pain, Z- disappearance of pain (Seconds)]																		

Exercise:

a. Explain the difference in the time of onset of pain in each procedure.

Explain the time required for the disappearance of the pain.

Explain the colour changes in the forearm during the exercise and on releasing the occlusion.

Explain any other sensations felt by the subjects.

Experiment C 5

PLETHYSMOGRAPHY

Plethysmography is a technique for measuring volume changes. This technique is used to measure blood flow to an organ. Occlusion of efflux of blood (veins) without affecting the influx increases the volume of the organ. This technique has a disadvantage in that the measurement should be made within a short period because the vascular bed of the organ will be filled with blood soon. The aim of this exercise is to measure the blood flow to fore arm under different conditions.

Instruments:

Plethysmograph, Volume recorder, Kymograph, Sphygmomanometer

Demonstration

The subject sits comfortably with the right arm inserted into the plethysmograph, which is placed on the table. A rubber sleeve seals the opening of the plethymograph around the arm. The rubber applied over the forearm should not compress the veins and cause venous obstruction before starting the experiment. One outlet from the plethysmograph is connected to a volume recorder, which writes on a moving drum of a kymograph.

A 50 ml syringe is connected to the other opening for calibration of the change in volume in the plethysmograph. Air is injected into the plethysmograph in varying volumes and the deflection of the pen for each volume is recorded.

The sphygmomanometer cuff is applied to the arm of the same limb and the blood pressure measured. This cuff occludes the veins of the arm when the pressure is increased to about 40 mm Hg (less than the diastolic pressure). Each recording begins as soon as the vein is occluded. The duration can be determined from the length of the tracing and the drum velocity. The rate at which the pointer rises is proportional to the blood flow. The rate of rising by the pointer slows down and the tracing flattens out as the veins are full and the blood flow has slowed down. Therefore the initial portion of the tracing only is used to calculate the flow.

The blood flow to the right fore arm is determined after the following procedures.

a. Resting blood flow

b. After keeping the left hand in warm water for 2 minutes (at about 45°C)

c. After keeping the left hand in cold water for 2 minutes (at about 10°C)

d. After subjecting the right forearm to ischaemia for four minutes.

e. After performing muscular work by the right forearm for four minutes.

f. After performing muscular work with arterial occlusion for four minutes.

g. After moderate work by the leg muscles on a cycle ergo meter for four minutes.

The mass of the tissue in the forearm is determined by displacement of water by the forearm up to the level that was inserted into the plethysmograph. Since the bones receive negligible blood supply the volume of the bones is deducted from the volume of the fore arm and flow/min/100g tissue calculated.

Observation

Stick the tracing

Calculation

Increase of volume and the duration are determined from the tracing. Inspect the tracing to observe the initial rising phase and a later stable phase when the veins have become full. The initial rising phase is extended by extending the tracing. The time is measured from the horizontal axis and the flow from the vertical axis.

If,

The increase in volume = V ml

The duration = T seconds

Flow in T sec = V ml

Flow in 60 sec = $\frac{60 \times V}{T}$ ml/min

Results:

a. Resting blood flow:……
b. After keeping the left hand in warm water :……
c. After keeping the left hand in cold water ……..
d. After subjecting the right forearm to ischaemia :…….
e. After performing muscular work:……
f. After performing muscular work with arterial occlusion……
g. After moderate work by the leg muscles on a cycle ergo meter:……

Exercise:

Explain the differences observed in the blood flow under difference conditions.

Experiment C 6

EFFECTS OF EXERCISE ON BLOOD PRESSURE
(Test for physical fitness – II)

The blood pressure and heart rate change during physical work along with respiratory and metabolic changes. The changes in circulatory system result in increased cardiac output and diversion of major portion of the cardiac output to the exercising muscles. This, along with other changes ensures increased supply of oxygen and essential nutrients to meet the increased needs of the working muscles.

This practical is designed to illustrate the changes in blood pressure and heart rate. These charges are brought about by nervous and chemical factors. The magnitude of the response and its duration after exercise of any individual will also depend on the state of the Autonomic Nervous system and the state of training to physical work. And this makes the experiment as a means of testing physical fitness.

Instruments:

Sphygmomanometer and cycle ergo meter or treadmill

Method:

Allow the subject to relax on the cycle ergo meter / treadmill and obtain the resting pulse rate and blood pressure. (Even though this is ideal, resting values may be obtained while the subject is seated on a stool in order to save time allowing another group to use the ergometer at this time.) Seat the subject on the ergometer, the blood pressure cuff still on but disconnected from the measuring device and start peddling at a rate of 30 cycles by each leg per minute (15 Km/hr) at moderate resistance (2 Kg). The rate of peddling is best kept with the help of a metronome. After four minutes of steady workout, stop and record the blood pressure and the pulse rate immediately and continue to measure every two minute until the resting values are reached. (Again, ideally the subject should be in the ergometer but for convenience, will have to move to a stool after the first measurement.

Demonstration:

Biopack system is used to demonstrate the change of pulse rate throughout the workout.

Observation

Name (Male)	Resting			After Exercise																
				0 minutes			2 minutes			4 minutes			6 minutes			8 minutes			Time taken to come to normal	
	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP		

Name (Female)	Resting			After Exercise															
				0 minutes			2 minutes			4 minutes			6 minutes			8 minutes			Time taken to come to normal
	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	

Exercise:

a. List the factors that influence cardiac and vasomotor centers and explain the relevance of each to physical exercise.

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b. Explain the charges observed during and immediately after the workout, in systolic and diastolic pressures and heart rate.

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c. Explain the mechanisms that bring the altered values to resting levels.

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d. Describe the cardiovascular adaptations seen in a person trained in physical work.

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e. Discuss the differences observed in the responses between trained and untrained persons.

Note: A physically fit person should have the measurements returned to resting values in less than eight minutes.

Experiment C 7

EVALUATION OF THE CARDIOVASCULAR SYSTEM

The aim of the practical is to illustrate the relevance of learning cardiovascular physiology theory and practical to clinical situation.

Functions of the cardiovascular system can be assessed either by clinical examination and or by investigations. All the results are put together to identify diseases and to decide on the management. All these are repeated in order to monitor the progress of the patient.

Clinical evaluation

First step in clinical evaluation is to obtain a detail history and analysis of the symptoms; this is followed by physical examination and analysis of the sings.

History:

Recording the history of the illness starts with inquiring the age, sex, occupation and the address of the patient. This, while helping to open the discussion, provides valuable information.

How does the above information help?

Ask the patient about the presenting illness. Cardinal symptoms relevant to cardiovascular system are pain or distress dyspnoea, orthopnoea and palpitation.

What are the physiological bases of the above symptoms?

List the cardinal symptoms of myocardial infarction, angina pectoris, intermittent caludication and Rayaud's phenomenon and explain the physiological basis of the symptoms.

Physical examination:

General examination

Observe in the face, skin, mucus membranes, feet and abdomen. Describe the changes that may be observed in the above and explain the physiological basis of them.

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Examination of the arterial pulse:

Feel the radial pulse and examine for rate, rhythm, character and volume. Feel the pulse at all pulse points and compare with the opposite side. Explain the physiological basis of changes in each of the pulse parameters.

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Explain the need for feeling the pulses in all points and comparing with the opposite side.

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Measurement of blood pressure

Explain the need for regular measurement of systolic and diastolic pressures.

Explain the value of the measurement in short term and long term management.

Examination of the jugular venous pulse

Observe the neck for pulsations, especially above clavicles, while the subject is seated. Get the subject to lie down in the bed and observe again. Place a finger gently above the clavicle and observe the change in the pulsation. Remove the finger and give a sudden push in the abdomen and observe the change in the pulsation.

Explain the origin and the waves of venous pulse.

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Is it visible in normal people?

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How does the venous pulse differ from arterial pulse?

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Explain estimation of central venous pressure.

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Discuss the significance of the central venous pressure in clinical situation.

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Examination of precordium

Inspection and palpation

Surface mark the heart and big vessels

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Locate the apex (it is the lowest and outer most point at which cardiac impulse is felt).

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Explain the factors that could shift the apex.

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Place the palm over the pericardium and feel for other pulsations and thrills. Explain the causes of other pulsation and thrills.

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Percussion

The middle finger of the left hand is placed firmly on the part to be percussed. The back of the middle phalanx is then struck with the tip of the middle finger of the right hand the quality of the sound depends on the nature of the structure. Solid structures and fluids (heart) produce dull sound and structures containing air (lung) produce resonant sound. Listen for any change in resonance.

How does percussion help in determining the size of the heart?

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Auscultation:

Describe the normal heart sounds and define the areas where they are best heard: mitral area – apex, tricuspid area – left of lower sternum, aortic area – right second intercostal space and the pulmonary area – left second intercostal space. Listen over the apex and other areas and identify the first and second sounds. Explain the physiological basis of changes in intensity, split in the sounds, audible third and fourth sounds, and presence of added sounds (murmurs and other sounds)

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Investigations:

Electrocardiography
Radiographic examination
External pulse recording
Phonocardiography
Echocardiography
Cardiac catheterization
Coronary angiography.

Experiment 8

ARTIFICAL RESPIRATION AND CARDIAC RESUSCITATION

When the heart or lungs fail for unnatural causes from which they can be revived, and the person is detected within five minutes so that there is no brain death, there is a good chance of reviving the person. As resuscitation in time makes the difference between life and death, every medical student should be skilled in delivering artificial respiration and cardiac massage.

Artificial respiration and / or cardiac massage should be delivered only if the organ concerned has completely failed. A model, Resusci Anne, is provided to practice these manoeuvres with an electrical system for guidance.

A hand out will be given at the session and trained tutors will demonstrate the procedure.

Exercise

a) Explain why mouth to mouth method is the best of all types of artificial respiration available.

Describe the risks of this method to the victim and to the operator.

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b) Explain the importance of correct hand position during cardiac massage.

Renal Function

Experiment K1

EFFECTS OF VARIOUS FACTORS ON FLOW OF URINE

When blood flows into the kidney, the plasma is filtered into the nephrons, required substances are reabsorbed, some substances are excreted and the remaining fluid with waste products flows into the ureters, then into the urinary bladder and out as urine. Urine flow is the volume of the urine that comes into the bladder from both kidneys in a unit time (ml/min). The flow depends on the glomerular filtration rate, osmolality of the medulla (counter current mechanism), the amount of the solutes in the lumen and the level of anti-diuretic hormone.

The aim of the practical is to demonstrate the effects of water, saline, frusemide, and dehydration on urine flow and its specific gravity. The specific gravity is measured as an index to the concentration of urine. At the end of the class, the students are expected to have understood the factors affecting urine flow and skilled in measuring the flow and specific gravity of urine.

Instruments:

Measuring glasses of different capacities, urinometer and beakers

The urinometer has a bulb and a shaft, the bulb has some heavy material to hold the instrument floating vertically when placed in liquids. The shaft is calibrated from 1.000 to 1.060 in steps of 0.020. The small divisions represent 0.002.

Method

The students are divided into groups and one male and a female from each group undergoes a particular procedure. All results are displayed on the board so that the students can compare and discuss the results of different procedures.

The subjects will go to the toilet and empty their bladder at zero time. They will go with a beaker and collect their urine in the beaker at the end of the next 30 minutes and hand over to the others in the group for measurements. The subjects should follow the specific procedure mentioned below. They will collect their urine every 30 minutes for the next 150 minutes making the total duration 180 minutes. The volume and specific gravity of each sample should be measured. If the volume is too low for measurements of specific gravity, the urine could be retained and added to the next sample for measurement of specific gravity. Also record the sensations of the subjects at different phases of each procedure, especially of thirst and preference to salt.

Group A: Control – 1

They form a control group. The subjects will only collect urine, every 30 minutes for measurements.

Group B: Control – 2

This also is a control group. The subjects will collect urine as others and will replace the fluid lost during the time by drinking water. 25 ml in excess of urinary output. (25 ml is to replace water lost by insensible perspiration).

Group C: Furosemide

The subject will receive an injection of furosemide (Lasix) 20 mg. intramuscularly after the first 30 minutes.

Group D: 1 L Water – 1 dose

The subject will drink one liter (1 L) of water after the first 30 minutes. They must drink the water slowly and steadily in less than 15 minutes.

Group E: 1 L water – 5 doses

The subject will drink one liter (1L) of water in five installments; they will drink 200 ml every 30 minutes.

Group F: 1 L Saline

The subjects will drink one liter (1L) of isotonic saline after the first 30 minutes. They must drink the saline slowly and steadily in less than 15 minutes. They should be careful not to provoke nausea or vomiting.

Group G: Dehydration

The subjects are different from the others in that they should have prepared themselves 5 hours ahead in order to dehydrate themselves. On the previous day they are identified and instructed to consume moderate amounts of fluids with breakfast and to take nothing after 8 A.M. on the day of experiment.

Observation

Group	Name	Sex	Urine	Time at which the samples were collected (minutes)						
				30	60	90	120	150	180	Total
A		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							
B		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							
C		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							
D		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							
E		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							

Group	Name	Sex	Urine	Time at which the samples were collected (minutes)						
				30	**60**	**90**	**120**	**150**	**180**	**Total**
F		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							
G		Male	Volume							
			Specific gravity							
		Female	Volume							
			Specific gravity							

Exercise:

1. Describe the formation of urine and the physiological mechanisms that affect the volume and specific gravity of urine.

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2. Outline the action of furosemide and explain the effect of the drug on urine flow.

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3. Draw graphs for urine flow and specific gravity with each procedure and explain the findings.

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4. Discuss the physiological mechanisms that are responsible for the changes in urine flow.

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5. Outline the relevance of the observations in day to day life and clinical practice.

Metabolism and Body Temperature

Experiment M 1

MEASUREMENT OF METABOLIC RATE

Every living organism depends on energy change from one from to another-metabolism - for its survival. Generally, chemical energy stored in the nutrients is converted into usable energy through ATP and finally the energy leaves the body as heat or external work. The rate at which energy is utilized, the metabolic rate, gives valuable information regarding the state of the body and the amount of food needed.

The metabolic rate can be measured either by measuring the heat output (direct calorimetry) or by measuring the oxygen consumption which is responsible for energy release (indirect calorimetry). Direct calorimetry requires enormous facilities to contain the individual in an insulated room over long periods and it is not possible in this laboratory. Two methods are available to measure metabolic rate by indirect calorimetry: spirometer method and Weir's method.

1. Spirometer Method

This is based on a subject breathing pure oxygen from a closed chamber and directing the expired air with remaining oxygen through soda lime to absorb the carbon dioxide and back to the chamber.

Instruments

Spirometer, oxygen analyzer, normogram for energy value of oxygen

Method

Empty the breathing chamber of the spirometer and turn the exit to "atmosphere" position. Fill the chamber with oxygen. Connect one tube leading from the exit the inlet of the one way valve; the out let of the valve to the lower opening of the soda lime canister; and the out let from the canister to the breathing chamber. Seat the subject comfortably and apply mouth piece and nose clip. Wait for a few minutes to till the subject familiarizes with the set up. Switch on the kymograph at low speed, turn the exit to "spirometer" position and wait for five minutes. At the end of the time stop the kymograph, disconnect the subject and remove the tracing.

Record the temperature in the breathing chamber, the atmospheric pressure at the barometer and the saturated vapour pressure at the temperature of the chamber. Obtain the energy value of the oxygen from the normogram.

Note that the normogram relates the energy value to the respiratory quotient which is the ratio of carbon dioxide produced to the oxygen consumed. As the carbon dioxide has been absorbed in the soda lime, actual respiratory quotient cannot be measured. The respiratory quotient, therefore, has to be deducted from the type of metabolic fuel, which depends mainly on the last meal. Assuming that the subject had a balanced meal, the respiratory quotient is assumed to be 0.85.

Observation

Stick photocopy of the tracing

Calculation

Draw a line to join the lower points of the tracing which gives the slope. Read the reduction of volume for one minute.

Assume,

Reduction of volume in one minute = V ml.
Atmospheric pressure = P mm Hg.
Temperature in the chamber = T.C
Saturated vapour pressure = W mm Hg.

First step in the calculation is to calculate the reduction of volume in one minute (Vs) at standard temperature and pressure.

$$\frac{760 \times Vs}{273} = \frac{(P-W) \times V}{(273 + T)}$$

$$Vs = \frac{(P-W) \times V \times 273}{(273+T)\ 760}$$

Vs x 60 ÷ 1000 = Oxygen consumption in litres per hour.

Now look at the normogram for energy equivalent oxygen at the RQ of 0.85 and calculate the metabolic rate.

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2. Weir's Method

This method is based on Weir's formula:

EE = (Oi – Oe) x Ve x 21.1 Kj/hr.

Where, Oi = oxygen fraction in inspired air,

Oe = Oxygen fraction expired air,

Ve = Volume of expiratory air in liters, and 21.1 is Weir's constant.

This method is easy because Oi is a constant atmospheric air) and Oe and Ve can be measured accurately. The volume should be in STP

Instruments

Douglas bag, Wet/Dry gas meter, oxygen analyzer (with drying agents)

Method

Take a Douglas bag and roll it empty and turn the tap so that it is closed and any air coming through the tube will go out. Seat the subject comfortably and connect Douglas bag through appropriate mouth piece, one way valve and tubing and apply the nose clip. When the subject is accustomed to the instruments, turn the tap and collect the expired air for five minutes.

At the end of the time, disconnect the bag and connect it to the gas meter (after bringing the pointers of the gas meter to zero) and roll the bag to empty the air trough the gas meter. Hold a thermometer in the outlet of the air stream to measure the temperature at which the volume is measured. Take a sample of the air and pass it through the Oxygen analyzer and measure the concentration of Oxygen. Record the atmospheric pressure at the barometer.

Calculation

Divide the Oxygen concentration by 100 to make it the fraction. Look at the saturated vapour pressure chart and get the vapour pressure at temperature recorded which will help to calculate the pressure of the dry expiratory gas. Convert the volume to STP and calculate the volume of expired air for one hour (Vs), Oi is a constant Oe is measured. Using these values, calculate the energy expenditure.

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Exercise

a. Explain the defect in the spirometer method as a result of assuming the respiratory quotient.

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b. Discuss the merits and demerits of both methods specially with regards to measuring the metabolic rate at different states such as basal, resting or exercise conditions.

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c. Outline the importance of measurement of metabolic rate for the community planning programs and in clinical situations.

Experiment M2

MEASUREMENT OF BODY TEMPERATURE

The core temperature is maintained around 37°C and shows a slight variation at certain instances: the diurnal variation, ovulation (in females), and physical exercise. When it is increased considerably (about 1° or more) the subject is said to have hyperthermia (pyrexia or fever) and when reduced hypothermia. Accurate measurement of the temperature is, therefore, very important. The surface temperature is usually less than the core temperature and depends on several physiological and environmental factors.

The core temperature is ideally measured in the oesophagus. For convenience it is generally measured in the mouth and at times it is measured in the rectum or in the axilla.

The main aim of the practical is training to record the oral temperature. The temperatures of the surface at specific points are also measured to illustrate the variation in surface temperature.

Instruments**:**

Clinical thermometer and laboratory thermometer

The clinical thermometer is specially made for measurement of oral temperature the bulb is made of thin glass and the lumen of the shaft is thinner than hair which has a constriction near the bulb. One surface of the shaft is painted white and the opposite edge is made into a lens to magnify the mercury column.

Method:

1. Seat the subject comfortably. Take a clinical thermometer, dip it in savlon, wipe it and wash it in cold water. Hold it tight and shake it so that the level of the mercury is far below 35°C. Place the thermometer under the tongue of the subject and ask the subject to keep the mouth closed. Take the thermometer after two minutes and read the temperature to the accuracy of at least one decimal point. (The mercury column is visible only in one angle and at a certain distance from the eye because of the lens). Wash the thermometer in the tap water and replace it in savlon.
2. Keep the bulb of the clinical thermometer in the axilla, cover the bulb with the arm for two minutes and record the temperature.
3. Keep the thermometer in the cubital fossa and flex the forearm so that the bulb is fully covered. Keep it for two minutes and record the temperature.
4. Keep the thermometer in the palm and cover the bulb by closing the fingers tightly for two minutes and record the temperature.
5. Keep the thermometer in popliteal fossa and flex the leg so that the bulb is fully covered. Keep it for two minutes and record the temperature.

Note: If in any of the above, the temperature happens to be below the measurable level of the clinical thermometer, and ordinary thermometer could be used. Then, it must be remembered that the temperature should be read while the bulb is in place and not after removing.

Observation

Name (male)	Oral	Axillary	Cubital	Hand	Popliteal
Mean					
SD					

Name (female)	Oral	Axillary	Cubital	Hand	Popliteal
Mean					
SD					

Calculation

Calculate the mean and standard deviation of the measurements for the class.

Exercise

a. Describe the special features of the clinical thermometer and explain their relevance.

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b. Explain the basis for keeping the bulb under the tongue and for closing the mouth for measuring oral temperature.

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c. Explain the possible errors that could arise in measuring the oral temperature.

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d. Discuss the merits and demerits or oral, rectal, axillary and oesophageal temperatures as measurement of core temperature.

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e. Explain the differences in the mean temperatures and standard deviation at different places and any apparent relationship to the distance of the place from the heart.

Neurophysiology

Experiment N1

EXAMINATION OF THE PERIPHERAL NERVOUS SYSTEM.

The peripheral nervous system performs sensory and motor functions. Examination of this system in a normal person demonstrates the physiological functions of the system and examination of a patient provides information regarding the exact location and the extent of any lesion and the nature of disability. The examination consists of tests for sensations, motor functions and reflexes.

Instruments:

Cotton wool, divider, tuning fork (128Hz), pin, hot and cold water in test tubes, and rubber (knee) hammer

Sensory system

All sensory modalities should be tested one by one all over the body represented by each dermatome, comparing the sensation of each side of the body at corresponding areas. When an area of deficiency is detected, the margins of the area of loss of sensation should be mapped by testing from impaired area to the normal area. Question like "do you feel now "should be avoided and the patient should be asked to respond positively. (E.g.-counting loudly every time he/she feels the sensation)

Tactile sensibility

Fine touch is tested with a wisp of cotton wool. The cotton wool should be placed on the skin gently without disturbing the hair. Crude touch is tested with the tip of the index finger. Ability to distinguish two points (two point discrimination) is detected with the help of a divider.

Position sense

Position sense is tested in all joints. The patient closes eyes or looks away. The joint to be tested is stabilized by one hand holding it by thumb and other fingers across the axis of movement to be tested. The distal part is flexed or extended by the other hand, holding on the sides, across the axis of movement to avoid the force used for movement giving any clue. The patient should respond when the position is changed by saying "up" or "down".

The dynamic appreciation of movement is tested by asking the patient to say "now" as soon as he/she feels the movement. Normally movement is felt within an angle of 10.

Recognition of size, shape, weight and form (stereognosis)

The patient is asked to close eyes or one hand is taken to the back and a familiar object is placed in the hand. The patient will have to feel and identify the object.

Vibration sense

The foot of a vibrating tuning fork is placed on the skin. Bony points are more sensitive to this sensation.

Pain sense

Superficial pain is tested with a pin but distinction should be made between pain sensation and fine touch. Pressure pain is tested by pressing hard or squeezing muscles.

Temperature sense

Temperature sense is tested with test tubes containing hot and cold waters. Adaptation of these receptors can be demonstrated by feeling water at room temperature after keeping the finger in hot or cold water for two minutes.

Exercise: Describe the change in sensation in various conditions- sensory nerve lesions, peripheral neuropathy, spinal cord lesions.

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Motor System

Bulk of muscles

Observe the muscle bellies and compare with opposite side, look for atrophy, hypertrophy, tremors and fasciculations.

Tone of the muscles

When the patient is completely relaxed move the limbs gently and feel the resistance to movement. Look for hypertonia or hypotonia.

Strength of Muscles

Ask the patient to force his limbs against resistance offered by you. Test the strength of each muscle separately and compare with the opposite side.

Coordination of Movement

Upper limb:

Ask the patient to touch the tip of his/her nose and then your finger. Then ask the patient to repeat it with eyes closed. Look for past pointing and shaky movement.

Ask the patient to tap on the table rapidly. Look for inability to perform rapid repeated movements (dysdiadochokinesia).

Lower limb:

Ask the patient to walk on a straight line.

Get the patient to a bed and ask to raise one leg in air, place the heel on the knee of the other leg and slide towards the ankle.

Exercise: describe the changes expected in upper motor and lower motor lesions, cerebella lesions and basal ganglia lesions.

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Reflexes

Reflexes have sensory and motor components. Reflexes are divided into two categories based on the type of sensation.

1. **Superficial reflexes:**

These are responses to sensations in the skin.

1.1. The planter reflexes:

Get the patient relaxed on a bed and stroke the outer edge of the sole of the foot, from heel towards the little toe, with a blunt instrument such as a key. In normal person planter flexion of the toes is seen (contraction of tensor fascia lata). Stronger stimulus produces dorsiflexion at the ankle and even withdrawn of the limb.

In pyramidal tract lesions the toes fan out and dorsiflex. This is positive Babinski response. This response with dorsiflexion of the ankle and flexion of the knee resembles withdrawal reflex to noxious stimulation.

Exercise: what changes occur in lesions?

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1.2. Abdominal reflex:

The patient is on bed with abdomen uncovered. General stroke of the abdominal skin towards midline causes contraction of the underlying muscles and manifests by pulling the umbilicus towards the stimulated area. This reflex is absent in upper motor neuron lesion.

Exercise: what changes occur in lesions?

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2. **Tendon (deep) reflexes:**

Tendon reflexes are monosynaptic reflexes where sudden stretch of the muscle spindles results in brief contraction of the muscle. This shows the integrity of the reflex pathway and excitability of the anterior horn cells. In order to produce sudden stretch the patient should be completely relaxed; the muscle should be slightly stretched by passive movement of the limb and a single sharp blow is given with a soft rubber hammer. The response is assessed by observing the sudden slight movement of the distal part or preferably observing the muscle belly contracting. Always compare the response with opposite side.

Exercise: What changes occur in reflex response in upper motor, lower motor, cerebella and Basal ganglia lesions?

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2.1. **Knee jerk:**

Get the patient in a bed, completely relaxed. Pass one hand under the knee and lift the joint a little. Strike the patellar tendon at the middle. This reflex can also be tested while the patient is seated up, longs dangling freely over the edge of the bed.

List the muscles and the spinal segments involved:

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2.2. **Ankle reflex:**

Bend the knee slightly and place the leg on the opposite leg. Hold the foot and dorsiflex the ankle slightly. Strike the tendon at the posterior surface and look for the response or preferably feel the force by the hand holding the foot.

List the muscles and the spinal segments involved:

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2.3. **Biceps jerk:**

Flex the elbow to a right angle and keep the forearm in a semiprone position. Place your thumb or index finger of one hand on the biceps tendon and strike over your finger. (Explain why biceps tendon was not stretched by extension of the elbow)

List the muscles and the spinal segments involved:

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2.4. **Triceps jerk:**

Flex the elbow and allow the forearm to rest on the chest of the patient. Strike the triceps tendon just above the olecranon.

List the muscles and the spinal segments involved:

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2.5. **Supinator jerk:**

Allow the forearm to rest on the chest of the patient in semipronation. Strike the styloid process of the radius.

List the muscles and the spinal segments involved:

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2.6. **Jaw jerk:**

Ask the patient to open the mouth slightly. Place your index finger firmly on the chin and tap on the finger as in percussion.

List the muscles and the spinal segments involved:

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Clonus:

Clonus is regular oscillations of contraction and relaxation. It is generally elicited in the ankle and some times in the knee. Sustained clonus occurs in upper motor neuron lesions. Unsustained clonus may occur in healthy persons who are tense or anxious.

Flex the ankle and support the leg with one hand. Dorsiflex the foot suddenly with the other hand and hold it dorsiflexed.

List the muscles and the spinal segments involved:

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SUMMARY OF REFLEXES

Superficial reflexes

Reflexes	Afferents	Center	Efferent
Corneal	Cranial V	Pons	Cranial VII
Nasal (sneeze)	Cranial V	Brain stem and Upper cord	Cranial V,VII,IX,X, & spinal
Pharyngeal and Uvular	Cranial IX	Medulla	Cranial X
Upper abdominal	T-7,8,9,10	T-7,8,9,10	T-7,8,9,10
Lower abdominal	T-10,11,12	T-10,11,12	T-10,11,12
Cremasteric	Femoral	T-1	Genetofemoral
Planter	Tibial	S-1,2	Tibial
Anal	Pudendal	S-4,5	Pudendal

Deep reflexes

Reflexes	Afferents	Center	Efferent
Jaw	Cranial V	Pons	Cranial V
Biceps	Musculo…	C-5,6	Musculocutaneous
Triceps	Radial	C-6,7	Radial
Periosteoradial	Radial	C-6,7,8	Radial
Wrist (flexion)	Median	C-6,7,8	Median
Wrist (extension)	Radial	C-7,8	Radial
Patellar	Femoral	L-2,3,4	Femoral
Achilles	Tibial	S-1,2	Tibial

Visceral reflexes

Reflexes	Afferents	Center	Efferent
Light	Cranial II	Midbrain	Cranial III
Accommodation	Cranial II	Occipital cortex	Cranial III, IV
Oculocardiac	Cranial V	Medulla	Cranial X
Carotid sinus	Cranial IX	Medulla	Cranial X
Bulbocavernosus	Pudendal	S-2,3,4	Pelvic autonomic
Bladder and rectal	Pudendal	S-2,3,4	Pudendal & autonomic

Experiment N2

EXAMINATION OF THE EYE

Eye is a special sense organ and its functions involve perception of light, image formation and transmission of impulses. The function is further carried out in the occipital cortex, association areas and finally in the Wernicke's area. These functions are facilitated by the eye muscles supplied by somatic nerves and cornea and ciliary body supplied by autonomic nerves. Complete examination of the eye, therefore, comprises several different procedures.

Experiment 2.1
Visual illusions

Several pictures are displayed for observation. Observe each picture from a distance and then read the notes given about the picture. This will help you to understand that vision is not just image formation but also interpretation in the cortex.

Experiment 2.2
Field of Vision

Gross abnormalities of the field of vision can be detected by clinical examination. Perimeter is used to map the field of vision accurately.

Clinical Examination for Field of Vision.

This is based on comparing the field of vision of the examiner with that of the subject, one eye at a time. The subject is seated in front of the examiner at about two feet, eyes of both at the same level. The subject covers that left eye and the examiner covers the right eye. Both fix the gaze of the uncovered eye on the other's eye. The subject is instructed to keep the eye fixed on the examiner's eye and to indicate when the moving finger of the examiner comes into view. The examiner moves the finger in the plane between the two. First the finger is raised up above the visual area and lowered slowly: when the subject reports of seeing the finger, the examiner will be able to judge the subjects upper quarter of the visual field by comparing with the personal perception. Similarly the finger is moved from nasal, inferior and temporal areas and the field of vision is tested. The procedure is repeated with the other eye.

Perimetry:

Instrument:

Perimeter is used to record the field of vision. One eye is tested at a time. A stand is provided to fix the chin and a white mark is placed in the instrument to fix the gaze. The height of the stand could be adjusted so that the tested eye is placed at the level of the mark.

A pointer moves in an arc by operating a wheel at the back. This pointer travels from the mark at the centre up to the vertical plane of the tested eye. The movement of the pointer is coupled to a pin at the back of the instrument. A paper is fitted to the paper holder and the position of the moving pin can be entered on the paper by pressing the paper holder against it. The whole assembly could be rotated through full circle the fields of vision at every angle could be detected accurately.

Method:

Take the perimeter chart and fix it on to its holder, so that the marks on the holder correspond to the marks on the chart. Move the pointer to the mark (bring the pin to the centre) and press the paper against the pin to make sure that the centre of the chart corresponds to the centre of the holder.

Seat the subject comfortably and cover one eye. Position the chin on its stand so that the eye to be examined is against the central mark on the perimeter. Instruct the subject to fix the gaze on the mark and indicate when the pointer comes into view. Take the pointer to the outer most point by rotating the wheel and move it slowly towards the centre. As soon as the subject gives the signal, stop the wheel and press the paper holder against the pin to get the position entered in the chart. Rotate the arc by 30° and repeat the procedure. Record the field of vision until the circle is completed.

Remove the chart and fit it again to test the other eye and carry out the procedure for the other eye. Remove the paper and join the points made by the pin to obtain the field of vision.

Past a copy of the Perimeter chart.

Give examples of change in visual field:

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Experiment 2.3
Measuring the diameter of the optic disc

This experiment demonstrates the blind spot in the field of vision and uses the blind spot to measure the diameter of the optic disc. The optic disc has no receptors on it and light is perceived by the retina around it. So, the diameter of the disc is proportional to the blind spot.

Method:

The subject stands about 150 to 200 cm away from a black board, one eye blind folded. A mark is placed on the board at the eye level of the subject and the subject fixes the gaze on the mark. The examiner moves a chalk from this point in the temporal fields of vision horizontally along the board. The subject is requested to signal when the chalk disappear from sight. As the signal is obtained, a mark is placed on the board at the point. The chalk is moved further, until it comes into view again. Another mark is placed on the board. The area between the two marks is the blind spot. The blind spot is mapped out by moving the chalk in and out of the blind spot at all angles and the circle is completed.

Measure the distance of the board from the eye, the distance of the medial edge of the blind spot from the mark and the diameter of the blind spot. The diameter of the optic disc and the distance of it from the visual axis is calculated using the principle of visual angle as follows:

The principal focal distance	= 1.7 cm.
Assume, that the distance of the subject form the board	= s cm
Distance of the blind spot from the mark	= v cm
Diameter of the blind spot	= d cm
Distance of optic disc from visual axis	= V cm. and
Diameter of the optic disc	= D cm

$$v/V = d/D = s/1.7$$

Calculate the Diameter of your optic disc and record it:……

Experiment 2.4
Measurement of intra ocular pressure

The aqueous humour of the eye is formed by the ciliary body, flows through the pupil and absorbed into the canal of Schlemm and from there to the venous plexus. The circulation of this fluid provides the pressure in the eye which helps to keep the spherical structure. When the formation is reduced as in severe dehydration the pressure reduces and when the drainage is affected as in glaucoma, the pressure increases.

The pressure can be clinically judged by pressing the eyeballs over the eyelid. The same principle is use to measure the pressure more precisely by a Tonometer.

Experiment 2.5
Visual acuity

The degree to which the details and contours of objects are perceived is the visual acuity. It is usually defined by the minimum separable distance between two lines. It is the shortest distance between two lines which can be identified as two lines. Defects in visual acuity may occur due to neural or refractive errors.

Snellen chart

Snellen chart is commonly used to test visual acuity. The letters on it are prepared so that the width of the lines in the letter subtend 1 minute arc and the minimum distance between the lines is also 1 minute and the width of the whole letter subtends 5 minute arc from the distances marked below them. An individual with normal visual acuity could read the first row of letters from a distance of 60 meters (200 feet). Similarly, the row of letters marked 6 meters (20 feet) could be read from that distance.

Method:

The snellen chart is placed at the eye level and illuminated well. The subject is placed 6 meters (20 feet) from the chart. One eye is blind folded. The subject is asked to read from top. The last line that is clearly read is noted. The procedure is repeated for the other eye.

The visual acuity is determined by dividing the distance from which the chart is read by the distance from which a normal person can read the last row read by the subject. If the subject has read the row marked 6 meters, his visual acuity is 6/6 (or 20/20). If the subject read up to the row marked 18 meters, the acuity is 6/18- less than normal. If the subject goes on to read the row marked 5 meters, then the acuity is 6/5 which is better than normal.

If the visual acuity of the subject is reduced, the test is repeated allowing the subject to read through a pin hole. The pin hole eliminates the function of the refractive mechanisms and the performance improves if the subject had refractive error. If the

reduction of visual acuity was due to neural problems, pin hole does not improve the acuity.

Test for near vision:

Jaeger's chart or Rayner test types chart is used. These are made of letters of different sizes. The subject starts at the smallest and goes on trying to read bigger letters. The chart should be kept at about 35 cm. from the eye.

Test for astigmatism

The chart with parallel lines radiating from a central point is observed by the subject. All lines will be alike if no astigmatism and certain lines will be blurred while others remain clear if the defect is present.

Exercise: list the visual defects and the lenses used to correct them.

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Experiment 2.6
Color Vision

Color vision is a complex function which involves retinal and cortical functions. Presence of three types of cones which respond maximally to blue, green and red (yellow) and the fact that these colours could be mixed to produce any color sensation forms the basis for the three primary colours. Even though the three types of cones respond maximally to specific colours, they respond at various levels to all light rays in the visual range of wave length. The final perception of color, therefore, appears to be the integration of the impulses from all cones by the cortical areas.

Demonstration of complementary colour:

Place a rectangular sheet of any color in well illuminated white background. Fix the gaze on the sheet for two minutes without moving the eyes. Remove the sheet of paper suddenly (preferably by another person). For a few seconds a different colour comes into view and fades off. This color is the complementary colour for the colour of th sheet. Repeat the test with different colours and determine the complimentary colour for all colours.

Test for colour blindness:

The cone pigments are essential for the perception of light by the cones. The gene for the blue sensitive cone pigment is on chromosome 7 and the genes for green and red sensitive pigments are on X chromosome. Because the red-green color blindness is sex-linked recessive characteristics, females are generally carriers and males are the sufferers.

Detection of colour blindness is not easy because those who are deficient in color vision has been also perceiving the rays and they were trained to call whatever the sensation they got as the color. Various tests have been developed to identify colour blindness.

Yarn-matching test:

This is a simple test. The subject is presented with a skein of yarn and asked to pick out the one which matches the colour from a pile of various coloured skeins.

Ishihara charts:

These are series of charts produced by tricky color dots. The subject is asked to read the number or trace the pattern in each chart and the interpretation of the response is given in a booklet.

Experiment 2.7
Eye Reflexes

The activities of the eye are effected by skeletal and smooth muscles which are supplied by somatic and autonomic nerves. Also several portions of the eye are rich in sensory nerves and several reflexes can be demonstrated.

Protective reflex:

Ask the subject to look at a distance object. Bring an object (may be your hand) rapidly towards the subjects eye. Observe the blinking of both eyes.

Corneal reflex:

Ask the subject to look ahead. Take a wisp of cotton wool and touch the cornea gently by a side without bringing the wool in to sight. Observe blinking of both eyes. Repeat the test by touching the conjunctiva and eyelashes.

Exercise: trace the pathway and describe the findings in possible lesions:

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Light reflex:

Ask the subject to fix the gaze on a distant object. Inspect the iris and identify the pupil of both eyes. Take a touch and shine it into one eye from one side, avoiding the visual attention on the torch. Observe constriction of the pupils of both eyes. Repeat the test with the other eye. As an alternative, observe the size of the pupil of one eye and then cover the other eye suddenly and observe the change in the size of the pupil.
Exercise: trace the pathway and describe the findings in possible lesions:
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Near Response:

The subject fist fixes the gaze on a far object while the examiner holds a illuminated object in front of the subject about 50 cm. away. The subject is then asked to look at the near object. Three important changes occur: convergence, pupillary constriction and accommodation. Convergence and pupillary constriction are easy to observe. Accommodation is difficult to observe. The images formed by reflection on the different surfaces are used to indicate accommodation.

When the subject is looking into distance and the object is held in front of the subject 3 reflections are seen: a clear, small upright image reflected from the cornea; a larger fainter upright image reflected from the anterior surface of the lens; and a small inverted image reflected from the posterior surface of the lens. Identify these images before asking the subject to look at the object and keep on observing the images while the subject does so. The first image does not change. The second image becomes smaller and moves towards the third image. The third image change very little.

Eye Muscles

The muscles of the eye keep both eyes focused on the same object at all times. This is shown by the position of the cornea. If the eyes are not parallel, it indicates presence of squint as a result of weakness or paralysis of one or more of the eye muscles.

This is further tested by moving a finger in front of the patient towards up, down, right, and left. Defects in the movement of any eye indicate paralysis or paresis of the muscle.

Experiment N3a

TESTS FOR HEARING

Hearing includes perception of sound waves in terms of pitch, amplitude and amber, higher functions such as recognizing and understanding the meaning of the words and the source of sound. This practical demonstrates the tests for perception of sounds only.

The receptors are in the internal ear and different areas in the basilar membrane respond to different frequencies. When sound waves reach the ear, they vibrate the tympanic membrane, which is amplified by the ossicles and transmitted to the fluid in the inner ear where appropriate receptors are stimulated: air conduction. Any vibration that travels through the bones of the skull will also be transmitted to the bony covering of the inner ear and to the fluid in it and stimulate the receptors: bone conduction. When there is defect in the external or middle ear, the air conduction is defective but hearing is possible through bone conduction. In defects of inner ear, hearings by both conductions fail.

Clinical Examination:

A simple clinical examination for hearing is to cover one ear and speak to the subject from behind (to avoid lip-reading) in low voice. Then repeat the same for the other ear.

Tuning fork test:

Tuning forks of different frequencies are used to test the whole hearing range of frequencies. Two different types of tests are available to test for hearing loss and to differentiate the conduction deafness from nerve deafness.

Rinne's test:

Strike a tuning fork and place its base on the mastoid process and ask the patient whether the sound is heard. If it is not heard, strike hard and repeat it. If it is not heard the subject is likely to have nerve deafness for that frequency. If it is heard hold it until the sound disappears. As soon as the subject indicates the disappearance of the sound hold the top of the tuning fork near the external auditory meatus. If the subject hears the sound, the ear is probably normal and if not the subject probably has conduction deafness. Repeat the test for that ear with tuning forks of varying frequencies to cover the audible range. Then test the other ear.

Weber's Test:

Strike a tuning fork and place its base on the vertex or at the centre of the forehead. Ask the subject whether the sound is heard in both ears equally or which ear is louder. If both ears are similar the hearing is normal. If one ear is louder than the other, the louder ear may have conduction deafness or the other ear may have partial or complete nervous deafness.

Audiometry

Audiometer is an instrument which has several facilities for objective assessment of hearing ability. It has two types of graded out-puts. One out-put goes to earphones for testing air conduction. The other output goes to a vibrator which can be fitted over the mastoid process for assessment of bone conduction. The instrument can generate pure tones at selected frequencies which covers the hearing range. Complicated inputs from tape recorders or conversation are also possible: this facility is not used for routine testing.

Method:

Seat the subject comfortably and switch on the audiometer. Turn the amplitude knob to the maximum. Initiate the sounds of pure tones and let the subject to familiarize with sounds of various frequencies. Then explain to the subject that during testing, the subject will have to indicate as the sound is recognized through the hearing aid.

Reduce the amplitude to the lowest level and fit the earphones. Select the output to one ear and select the lowest frequency. Switch on the sound generating mechanism and go on increasing the amplitude slowly. As the subject signals of hearing the sound, note the amplitude and enter it on the record sheet against that frequency. Repeat the procedure with the next frequency until all are tested.

Then perform the test for the other ear and follow it with test for bone conduction on both sides. At the end a record of minimum amplitude of the sound heard by the subject at each frequency by each ear through air and bone conduction is made out.

Exercise: Describe the findings in possible lesions:

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Experiment N3b

TESTS FOR CHEMICAL SENSES

Taste and smell are components of chemical senses. Even though the receptors, their location, and the nervous connections are different for both sensations, perception of both is interconnected and provides the flavor of the food. The sense of smell in humans has apparently lost its importance in identification of substances for protection and sexual function.

The aim of this practical is to demonstrate the senses of taste and smell, the ability to identify substances by these senses. Also, the precautions to be taken to test these sensory modalities to locate nerve lesions are highlighted.

Method:

A series of solutions of various substances are placed on one side of the lab. Get one of your companions to deliver one drop of one solution on your tongue by means of the dropper given. Record the taste modality (sweet, sour, salt, or bitter) and the substance dissolved against its number. Wash the mouth thoroughly before testing the other substance and go on to complete the series.

Another series of substances are placed on the other side. Close your eyes, take the bottle near the nose and sniff gently. Identify the smell and note the name of the substance against its number. Complete this series.

The identification can be corrected with the list available with the staff member.

Note:

Hot is not a taste modality as it stimulates the pain fibers and not the taste buds. Ammonia, peppermint and other irritants also stimulate pain fibers in nasal cavity and they do not stimulate sense of smell.

Exercise: list the causes of loss of these sensations:

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Experiment N4a

VISUAL AND AUDITORY EVOKED POTENTIALS

(Demonstration)

Evoked potential is the recording of neural responses following the presentation of an external stimulus. Evoked responses can be quantified by measuring peak amplitudes and latencies. This is one of the most effective methods for examination of CNS functions. Visual Evoked Potentials and Auditory Brainstem Potentials are demonstrated.

Instruments

Neuropack, electrodes, Skin pure gel

Visual Evoked Potentials (VEP): pattern reversal stimulation

When visual stimulation is given by altering black and white checked pattern, responses to this reversal can be recorded from three electrodes spanning the occipital region with mid frontal electrode as voltage reference. This evoked potential is triphasic (negative – positive - negative). The three peaks in this VEP are N75, P100, and N145 (Positive or Negative, milliseconds). It is believed that the N75 is generated in the visual cortex; P100 potential with the peak latency of about 100 ms is generated in the extrastriate cortex of the middle occipital gyrus; and N145 is generated in the deep sources of the occipital cortex. VEP is useful in detecting lesions like demyelination of optic nerves, optic neuritis, and optic neuropathy and cortical blindness by evaluating the amplitude and the latency.

Method

1. Clean the subject's skin (sites where the recording and ground electrodes are attached) using cotton moistened with alcohol and then rub the skin with Skinpure skin preparation to reduce the artifact.
2. Attach the recording and ground electrodes on the subject.
3. Place the monitor at 1 meter form the subject.
4. Make the examination room dim.
5. Ask the subject to concentrate and watch the square at the center of the screen without blinking.
6. Get the average response in the occipital region with Neuropack.

Auditory Brainstem Potentials

When an auditory stimulation is presented to an ear through a head phone, an evoked response can be recorded by electrodes fixed on the vertex (+) and earlobes (-). The response wave has 6 - 7 positive potentials and it appears within 10 ms after stimulation. It is called **Auditory Brain Response (ABR)** or **Brainstem Acoustic Evoked Potential**

(BAEP). These waves are named I- VII in the order of appearance. They are generated in the auditory pathway between VIIIth nerve, brainstem and midbrain.

Clinically ABR is used to detect neurological and audiological abnormalities. In neurological abnormalities, latency, amplitude and inter- peak latencies are useful in localizing the lesions. Performing the ABR stimulation in series of intensities will detect audiological abnormalities. Smaller response at all intensity levels are observed in conductive deafness. In sensorineural loss, smaller responses are observed at low intensity level and normal responses are obtained in high intensity level.

Method

1. Clean the skin using cotton moistened with alcohol and then rub the skin with Skinpure skin preparation to reduce the artifact and attach the recording ground electrodes.
2. Attach the recording and ground electrodes to the optimal site on the patient's head.
3. Put the head phone on the patient.
4. Prevent all outside noises (conduct in the sound proof room).
5. Mask the opposite ear when stimulation is applied to one ear.
6. Record the ABR.

Experiment N4b
REACTION TIME

We react to changes in the environment. The changes in the environment are perceived through the sense organs, the stimuli are analyzed in the association areas, appropriate course of action is decided at the Wernike's area and appropriate motor activity is planned and executed. All these activities require several synaptic activities involving several neural circuits. The time taken from the occurrence of the stimulus to the execution of the task is the reaction time.

Instrument:

A program has been developed to measure the time elapsed in milliseconds in a computer clock. Pressing the space bar in the first instance will deliver a visual or auditory stimulus as previously selected. Once the stimulus is perceived, the subject will press the space bar as soon as possible. The computer will display the time elapsed between the occurrence of the stimulus and the pressing of the space bar and that will be the reaction time.

Method:

Seat the subject in front of the key board comfortably. The blue line message on the screen will indicate whether the selected stimulus is visual or auditory. Pressing 'A' will change it to 'auditory' stimulus and pressing 'V' will change back to visual stimulus. After selecting the stimulus, the subject will press the space bar to activate the timer.

- The timer will change the colour of the message box to green in 5-10 seconds in visual mode. In auditory mode, a buzzer will be sounded (ensure that the volume is set at maximum to ensure audibility).
- The subject should press the space bar as soon as the stimulus is detected.
- The computer will display the reaction time as the 'time elapsed'.
- Three attempts are allowed for each stimuli and the minimum reading will be the reaction time.

The same procedure is repeated while the subject counts backwards from 1000 loudly. This will demonstrate the effect of distraction on reaction time.

Results:

Name(Males)	Normal		Distraction		Name(Females)	Normal		Distraction	
	V	A	V	A		V	A	V	A
Mean									
SD									

V- Visual, A-Auditory

Discussion:

Discuss the importance of reaction time in driving vehicles and the effects of distraction and alcohol on driving.

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