PHYSIOLOGY PRACTICAL WORKBOOK

For Medical, Medical Sciences, Nursing Science, Physiotherapy, Radiography, Pharmacy, Optometry and Vision Science, Biomedical Engineering, Home-Economics, Performing Art & Other Health-allied Courses

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P R E F A C E

The Department of Physiology is pleased to present this revised edition of the Physiology Practical Manual, developed to support the teaching and learning of physiology across the various programmes within the College of Health Sciences and other related disciplines. This manual serves as the approved guide for practical sessions undertaken by students of Medicine and Surgery, Physiology, Anatomy, Nursing Science, Medical Laboratory Science, Physiotherapy, Pharmacy, Radiography, Biomedical Engineering, and other Health Allied Science programmes.

Physiology is fundamentally an experimental science, and a sound understanding of its principles requires more than classroom instruction. Practical sessions provide students with the opportunity to observe, investigate, and apply physiological concepts through hands-on laboratory experience. This manual has therefore been carefully designed to bridge the gap between theoretical knowledge and practical application, enabling students to develop the scientific, analytical, and technical skills expected of future healthcare professionals and biomedical scientists.

Within this manual, students will find clearly structured practical exercises, learning objectives, experimental procedures, recording templates, laboratory safety guidelines, and questions designed to stimulate critical thinking and reinforce key physiological concepts. It is intended to serve not only as a laboratory guide but also as a valuable companion for revision, self-directed learning, and preparation for practical examinations.

In line with the current National Universities Commission (NUC) Core Curriculum and Minimum Academic Standards (CCMAS), this manual promotes competency-based learning and encourages accuracy, professionalism, teamwork, ethical conduct, and adherence to laboratory safety practices.

It is our sincere hope that this manual will enhance the quality of practical instruction, stimulate scientific curiosity, and inspire students to appreciate the vital role of physiology in understanding normal body function, disease processes, and clinical practice. We are confident that the knowledge and practical skills acquired through this manual will

contribute significantly to the development of competent, innovative, and compassionate healthcare professionals.
The Department remains committed to the continuous improvement of teaching, research, and practical training. We therefore welcome constructive suggestions that will further enhance future editions of this manual.

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GENERAL NOTES AND INSTRUCTIONS

The purpose of the practical classes is fourfold:

1. To enable you learn some of the practical techniques used in clinical investigations. This is not necessarily the same as acquiring technical skill, which comes only with much repetition and practice.
2. To enable you appreciate physiology as an experimental science and as such requires a scientific and methodical approach.
3. To re-encode the physiological knowledge you have gained from lectures and from reading by direct observation.
4. To give you an opportunity of discussing physiological problems with members of the teaching staff and demonstrators.

HOW TO BENEFIT FROM THE COURSE

1. COME PREPARED to all practical classes. This means (i) make sure you know beforehand which experiment you are going to perform and read it up in the schedule. (ii) Go through it again with the apparatus in front of you in the laboratory before beginning the experiment. (iii) Bring everything you are likely to need for Physiology experiments. These include dissection instruments and laboratory coats which should be brought to every class whether or not the experiment indicates the need. It may sometimes be necessary, at the last moment, to change the practical schedule.
2. Participate as much as possible in the experiments. It will rarely be possible for experiments to be performed in pairs (the ideal number for most experiments) and you will usually find yourself a member of a larger group. This should not mean that only two members of the group should do all the work while the others are merely spectators, or worse still, sit reading newspapers or magazines. Offer active help and/or take it in turns to perform various parts of the experiment.
3. The demonstrators are available to give assistance when necessary and not to perform the experiment (unless it is a demonstration). Ask for their help when you are genuinely in difficulty and not because you can't be bothered to read the schedule.
4. BE PUNCTUAL so that the group introductions to the experiments are completed in good time, leaving as much time as possible for your own experiment.
5. When performing the experiment, make sure you obtain ALL NECESSARY data before leaving the laboratory. Ensure tracings are fully labeled. Such labeling should include heading, date, time. Trace arrows indications, e.g. addition of drug, which should be labeled with name of drug, its concentration and any relevant information. When you work in groups make sure you have your own set of data and when only one kymograph tracing is available, copy or trace the recording for your own use.

USEFUL HINTS IN WRITING UP EXPERIMENT

Submission of Workbook

Your practical schedule/workbook, marked after every practical session, will form part of the overall assessment of your practical work.

Successful experimentation consists not only in accurate observation and recording of data, but also in correct interpretation of results. Do not write up your practical schedule in the laboratory. You can use a rough notebook or loose writing material for making notes in the laboratory during the experiment. Answers to each experiment should be written up in your practical workbook after the experiment as soon as possible.

Practical Notebook

In addition to this manual and workbook, each student is required to have a practical notebook. These should be large notebooks with stiff covers. It is NOT necessary to buy the type with alternate graph paper. The write-up should normally take the following format:

Title or object of the experiment (with date).

Method: This should not be a repetition of the practical schedule. If the experiment was performed exactly as in the schedule, then it is sufficient to state that this was the case. However, in many experiments you may find you had minor modifications or it may be useful to add some hints and diagrams etc. to make the printed sheets more meaningful.

Results: This should, when possible, be neatly tabulated, calculations shown in full, graphs plotted and labeled where necessary, tracings should be cut into small portions to illustrate the relevant points and stuck firmly into place.

In expressing you results, be sensible. In general, don't put more significant figures into a calculated value that is contained in the measurements themselves. For example, you may analyze room air and calculate from the results that the percentage of oxygen is 21.782%. However, you will know that with the method you have used, coupled with your own relative inexperience in handling the apparatus, it would be totally unrealistic to assume that you could possibly be as accurate as this. A more realistic expression of the result obtained would, therefore, be 22%.

If you are unlucky and do not obtain any results, you may say so; but you may also find it useful to obtain results from a colleague so that you would know how to handle such results if they are presented to you in an examination.

Conclusion and/or Discussion: Any relevant conclusion should be clearly stated. In addition, the results should be fully discussed, including such points as whether or not the results are normal. If not, why not? What physiological mechanisms are involved?

Class Sheets

Class sheets may be provided for some of the experiments so that all the results obtained by the group may be collated. This may serve to introduce some of the concepts of medical/biostatistics and to demonstrate how "normal" your results are related to the rest of the class. The overall class result will be given to you usually in your next practical class.

NOTES ON INSTRUMENTS

Ideally for physiological experiments, instruments that are used for anatomical dissections should not be used for living tissues. Since it is realised that it is uneconomical for students to purchase two sets of instruments, it is essential that your instruments should be washed very thoroughly after anatomy dissection classes.

The following represent the minimum necessary instruments you will need:

Scissors: Two pairs (a) a rough pair of medium size, useful for cutting through bone, skin etc. (b) a fine, sharp pair that cuts right to the points of the blades.

Forceps: Two pairs (a) fine pointed forceps, (b) blunt-ended medium forceps. A pair of fine curved forceps may also be used.

Scalpels: Will not normally be used for experiments you will perform yourself.

Dividers: A pair of dividers and a ruler is useful for making accurate measurements.

CONDUCT IN THE LABORATORY

1. Laboratory coat should normally be worn.
2. All bags should be left outside or near the door of the laboratory.
3. There should be no eating, drinking or smoking in the laboratory (unless this forms a part of the experiment).
4. Your own bench must be left tidy at the end of the class. All dead animals and tissues should be put in the bucket provided.
5. Any breakage or faults in apparatus must be reported to the technical staff.

GENERAL RULES FOR WORKING WITH LIVING TISSUES

In carrying out experiments on any living tissue, certain elementary rules must be followed:

1. Ensure that the apparatus is completely ready and in working order before preparing the tissue. Even the most robust tissue deteriorates after removal from the animal and the longer it is before carrying out the experiment the poorer your results are likely to be.
2. An important part of preparing the apparatus is to wash your instruments thoroughly, even if they were washed after the last experiment, as they should have been. Particular care is needed if the instruments have been used in anatomical dissections of preserved materials.
3. Wash your hands thoroughly immediately before beginning dissection and after any dirty job during the practical, e.g. smoking a drum, or varnishing a tracing. In experiments with living tissue a high degree of cleanliness is essential.
4. Living tissues must be kept moist with the appropriate physiological saline. Any tendency to dry up will lead to irreparable damage and poor results. Handle the tissue very carefully,

especially nerves. A nerve damaged by pinching or stretching will not survive to conduct an impulse well.

5. In carrying out dissections, it is best to sit down at the bench so that the wrist can be rested on the bench. The hands can then be held much steadier and better dissection will be attained.
6. Take great care of your instruments. Protect the points of your scissors and forceps. Keep your fine scissors for fine work; do not attempt to cut bone, skin or any other tough tissue with them. At the end of the experiment, wash and dry instruments thoroughly to avoid corrosion.
7. When pin electrodes are being used they should always be placed carefully in contact with the tissue to be stimulated, so as to deform it as little as possible. Make sure that they are fixed independently of the tissue, so as not to drag on it, and see that they are not submerged in a large pool of saline which will short-circuit part of the current intended for stimulating the tissue. Do not push pin electrodes down on top of a nerve. This crushes it.

PREPARATION OF ANIMAL

The actual type of preparation required depends partly on the animal being used and partly on the type of experiment to be performed.

1. If the experiment is to be performed *in vivo,* the suitable anaesthetic must be used to ensure that the animal is unconscious and yet remains alive for the duration of the experiment. The anaesthetic use and its mode of administration is usually given at the beginning of the appropriate procedure.
2. If the experiment is to be performed *in vitro*, i.e. outside the animal, then it may be adequate to kill the animal by a sharp blow on the back of its head, the tissue then being dissected out from the animal with maximum speed, particularly in the case of mammalian tissues.
3. In the case of toad experiments, the animal is prepared in various ways depending on the nature of the experiment to be performed. In all of them the FOREBRAIN is destroyed so that the animal does not feel pain.

Destruction of the entire nervous system

This is the commonest preparation. Stun a toad by striking the back of its head by placing the lower blade of a strong pair of scissors between the jaws and the other blade over the dorsal surface of the skull. With one clean out along a line joining the interior borders of the tympana, remove the anterior part of the skull containing the cerebral hemisphere and mid-brain. At this stage, the preparation is a decerebrate preparation. Now, push the blunt seeker down the vertebral canal, thus destroying the rest of the C.N.S. i.e. the medulla and spinal cord. The entire C.N.S. is now destroyed, all reflexes are abolished, and the preparation is said to be "pithed". A twisting movement of the seeker effects the necessary destruction. As the central nervous system is being destroyed, there is stimulation of the body and limbs. Make sure that the animal is now without reflexes.

Decerebrate Preparation

Destroy the forebrain as already described. After recovery from shock, the preparation has normal reflexes.

Spinal Preparation

Stun a toad and pass the end of a scalpel handle lightly backwards over the dorsal surface of the head until the occipito-atlentoid joint is reached. This is recognised as a depression just posterior to the slight protrusion formed by the occipital condyles. Insert the point of the scalpel into the joint and sever the spinal cord. Then bend the skull forward and pass a blunt seeker through the opening just made into the cranium so as to destroy the brain. Cut off the front of the head as before, to make certain the forebrain is intact after a short interval, the spinal reflexes can be readily obtained.

Medulla Preparation

Stun a toad and cut open the occipito-alantoid joint as for a spinal preparation. Insert a blunt seeker and pass it downwards destroying the spinal cord. Now decebrate the preparation by cutting off the front of the head. The medulla is thus left intact.

PHYSIOLOGICAL SOLUTIONS

Isolated organs and tissues will only remain alive outside the body if they are surrounded by the current physical and chemical environment, i.e. if immersed in a solution containing the correct concentration of salts, within the permitted range of temperature and pH and an adequate oxygen supply.

Correct in this context means that the solution should, as far as possible, resemble that of its normal environment within the body.

The composition of such solutions varies for different animal groups and for different tissues. Some of the common physiological solutions, which you will encounter during your practical courses, are shown below:

TOAD TISSUES

Isotonic Saline: 0.6% NaCl (pprox. 150 mEq/l).

Ringer's solution:

NaCl	–	0.6%
KCl	–	0.042%
$CaCl_2$	–	0.024%
$NaHCO_3$	–	0.015%
Glucose	–	0.1%

MAMMALIAN TISSUES

Normal (i.e. isotonic) saline: 0.9% NaCl (pprox. 150 mEq/l).

Heart-Ringer-Locke's solution:

NaCl	–	0.9%
KCl	–	0.042%
$CaCl_2$	–	0.024%
$NaHCO_3$	–	0.015%
Glucose	–	0.1%

Intestinal Tyrode's Solution:

NaCl	–	0.8%
KCl	–	0.02%
$CaCl_2$	–	0.02%
$NaHCO_3$	–	0.1%
$MgCl_2$	–	0.06%
NaH_2PO_4	–	0.005%
Glucose	–	0.1%

EXPERIMENT 1

OSMOTIC AND PERMEABILITY PHENOMENON IN ERYTHROCYTES

I. EXAMINATION OF BLOOD CELLS AND WATER MOVEMENT DUE TO OSMOSIS

QUESTION

Why does citrate prevent blood clotting?

1. Place 1 – 2 mls of 0.9% solution of sodium chloride in a test tube and transfer it to a small quantity of citrated blood. The final mixture should be pale red in colour. Place one drop of the diluted blood on a microscope slide cover with a coverslip and examine under the microscope using first low and then high power. Observe the appearance of the erythrocytes and note that the thicker rim of the biconcave disc can be shown by focus up and down. Examine the slide carefully for leukocytes, which are slightly larger than the erythrocytes and appear granular on focusing up and down. You will have to search thoroughly because there are approximately 80 erythrocytes to one leukocyte.

2. Now place a drop of distilled water at one edge of the coverslip so that it can run underneath. To encourage this, hold a piece of filter paper at the opposite edge of the coverslip so that the fluid is drawn through. Examine the slide under the microscope where the distilled water mixes with the cells. Observe and note any change.

3. Make similar observations mixing the cells with 2% NaCl taking a fresh drop of the diluted blood in 0.9% NaCl for each observation.

II. FRAGILITY OF RED CELLS

QUESTIONS

1. In which tube(s) is the salt solution just slightly coloured with haemoglobin?

_____

2. In which tube is haemolysis complete?

_____

III. THE EFFECT OF SUBSTANCES WHICH PENETRATE THE CELL MEMBRANE

QUESTION

Explain why 'isotonic' solutions of urea and thiourea should cause the red cells to swell and finally haemolysed.

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(a) Haemolytics (Demonstration only)

QUESTION

Observe and note what happens in each tube.

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EXPERIMENT 2

TO DETERMINE THE NUMBER OF RED CORPUSCLES PER CUBIC MILLIMETRE OF BLOOD

QUESTIONS

1. What is the normal range of RBC count in male and female students in your practical group?

2. List four factors that can lead to physiological variations in RBC counts.

3. Discuss the possible errors of the method.

4. What are the functions of the different ingredients of the diluting fluid?

5. Discuss the clinical significance of a red cell count.

EXPERIMENT 3

TO DETERMINE THE NUMBER OF WHITE BLOOD CORPUSCLES PER CUBIC MILLIMETER OF HUMAN BLOOD

DIFFERENTIAL WHITE CELL COUNT

QUESTIONS

8. What is the normal range of WBC count in male and female students in your practical group?

9. List four factors that can lead to physiological variations in WBC count.

10. What is the ratio of WBC to RBC count?

11. Discuss the possible errors of the method.

12. What are the functions of the different ingredients of the diluting fluid?

13. Discuss the clinical significance of a white cell count.

14. Why will this experiment not be effectively carried out with bank blood?

EXPERIMENT 4a

HAEMOGLOBIN ESTIMATION

QUESTIONS

1. What was the mean value of the haemoglobin concetration obtained in your group.

   (a) Sahli's method.

   _____

   (b) Colorimetric method.

   _____

2. Discuss the possible errors of the two methods.

   _____

   _____

   _____

   _____

   _____

   _____

EXPERIMENT 4b

ESTIMATION OF PACKED CELL VOLUME (HAEMATOCRIT)

QUESTIONS

1. What cells form the uppermost layer of the packed cell column?

2. (a) Do you expect the haematocrit of the blood in capillaries and small vessels of the body to differ considerably from that in large vessels?

   (b) Why?

3. Discuss the significance of the use of the various parameters which you have obtained.

Use extra sheets if necessary

EXPERIMENT NO. 5

EXPERIMENTS ON HAEMOSTASIS

This practical class will take the form partly of a demonstration

WHOLE BLOOD COAGULATION TIME

At what stage in the clotting process does each of these substances act?

PROTHROMBIN TIME

QUESTIONS

1. Discuss the difference between bleeding time and coagulation time.

2. On what factors do they depend?

EXPERIMENT 6

DETERMINATION OF BLOOD GROUPS

QUESTIONS

1. (a) Why is it necessary to use only types anti-A and anti-B sera for determining blood groups?

(b) What is meant by anti-A and anti-B tying sera?

2. How are blood groups used to establish parentage in legal medicine?

3. Transfusion of mismatched bloods may cause kidney failure. Explain.

4. How do you account for the condition of anaemia found in *erythroblastosis fetalis*?

EXPERIMENT 6b

ERYTHROCYTE SEDIMENTATION RATE IN MAN

QUESTIONS

1. The normal human adult male ESR is 2 – 8 mm/hour while in adult female it would be between 2 – 12 mm/hour. Why is the value very often higher in females?

2. What factors influence the sedimentation of RBC.

3. List precautions you would take to ensure accurate results.

4. Why is it required to have a perfect vertical position and to take the ambient temperature during ESR measurement?

_____

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SECTION II. CARDIOVASCULAR SYSTEM

Experiment 8a: The cardiac cycle in the toad.

8b: To study some of the properties of cardiac muscle.

8c: Further properties of cardiac muscle.

Experiment 9a: Perfusion of the mammalian heart.

9b: Demonstration of some cardiovascular and respiratory reflexes and some factors which may affect them.

Experiment 10: Physiological properties of cardiac muscle: The ventricular function curve.

Experiment 11: Human Circulation.

(a) Examination of the heart.

(b) The pulse and study of the veins.

(c) Sphygmomanometry.

(d) Factors affecting the pulse rate and blood pressure.

Experiment 12: Recording of human electrocardiogram.

Experiment 13: Measurement of blood flow in a plethysmograph.

EXPERIMENT 8a

THE CARDIAC CYCLE IN THE TOAD

BEFORE BEGINNING THE EXPERIMENT REVISE THE INTRODUCTORY NOTES ON WORKING WITH LIVING TISSUES

1. RECORDING THE NORMAL RHYTHM OF TOAD HEART IN SITU

(i) Determine and record the frequency of contraction below:

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_____

_____

_____

_____

_____

II. THE EFFECT OF DRUGS ON THE ACTIVITY OF THE TOAD HEART

Explain the results obtained in relation to muscle activity and membrane potentials.

_____

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EXPERIMENT 8b

TO STUDY SOME OF THE PROPERTIES OF CARDIAC MUSCLE

QUESTIONS

1. Define the 1st ligature.

2. (a) What is the staircase phenomenon?

   (b) Discuss the possible explanation of the staircase effect particularly in relation to the "All or None" law.

3. Calculate the latent period when the heart is mechanically stimulated and compare with that obtained by electrical stimulation.

EXPERIMENT 8c

FURTHER PROPERTIES OF CARDIAC MUSCLE

QUESTIONS

1. Try to explain your results in terms of the absolute and relative refractory periods of cardiac muscle.

This method can be used to give a rough indication of the refractory period of cardiac muscle.

2. Calculate the refractory period obtained by this method and compare it with that obtained by a more accurate method described.

I. MEASUREMENT OF REFRACTORY PERIOD OF CARDIAC MUSCLE BY METHOD OF APPROXIMATION OF STIMULI

QUESTION

Discuss the advantages/disadvantages of this method compared with the method used in I.

II. SUMMATION OF SUBLIMINAL STIMULI

QUESTION

At approximately what frequency does the summation of subliminal stimuli occur?

_____

_____

_____

_____

GENERAL QUESTIONS ON EXPERIMENTS 8a, 8b AND 8c

1. The heartbeat may be described by its rate, rhythm and force or amplitude. Describe the heartbeat of the toad as best as you can.

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_____

2. What is the effect of temperature on the heartbeat?

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3. Explain why an extrasystole is followed by a compensatory pulse.

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4. What is the amplitude of the extrasystole like compared to the normal beat?

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5. What conclusions can you make from the stannous experiment?

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_____

6. From the “all or none” contraction experiment, explain why cardiac muscle appears to differ from skeletal muscle.

7. Outline the mechanisms of action of acetylcholine and adrenaline on the heart.

EXPERIMENT NO. 9

PERFUSION OF THE MAMMALIAN HEART
(DEMONSTRATION OF THE LANGERDOFF'S METHOD)

QUESTIONS

1. Discuss the physiological significance of the results obtained.

2. What are the effects of (a) adrenaline, (b) calcium ions, and (c) potassium ions on cardiac contractility?

3. What are the effects of excess calcium and potassium ions on the heart. Give the mechanisms of their action on the heart.

EXPERIMENT NO. 10

HUMAN CARDIOVASCULAR SYSTEM (I)

EXAMINATION OF THE HEART

QUESTIONS

1. Describe the heart sounds and murmurs that may be heard in disease conditions.

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2. What can you hear of the heart sounds when listening over the carotid artery?

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THE PULSE

The subject should sit at rest for five minutes so that any disturbance due to activity or emotion may pass off.

The radial pulse is most conveniently felt when the subject's arm is pronated. To feel the pulse, place three fingers of the right hand on the subject's radial artery at the wrist.

Make out the size of the vessel and condition of walls and observe:

(1) Rate: Count, record the number of beats in one minute.

_____

_____

(2) Rhythm: Do the pulsation follow at regular intervals, i.e. is the rhythm regular?

_____

_____

In some subjects, it may be found that the heart rate increases slightly during inspirations. This is called sinus arrhythmia. Are all the pulsations of equal force?

_____

_____

Volume: The volume or amplitude of the artery can be noted by light palpation; estimate it as large, medium or small.

_____

_____

QUESTIONS

1. Discuss the physiological importance of the presence of valves in the veins.

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2. Why are they not essential in the arteries?

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EXPERIMENT NO. 11

HUMAN CARDIOVASCULAR SYSTEM (II)

TO INVESTIGATE SOME FACTORS WHICH AFFECT PULSE AND BLOOD PRESSURE

Before beginning this experiment, make sure that you are fully conversant with the methods described in Experiment No. 8 so that every member of the group can quickly and accurately measure pulse rate and blood pressure. Practice is the only way this can be achieved.

QUESTION

Record the results of the experiments graphically where possible. Explain the physiological mechanisms of the observation you have made.

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Use extra sheet(s) if necessary

EXPERIMENT NO. 12

MEASUREMENT OF BLOOD FLOW WITH A PLETHYSMOGRAPH (DEMONSTRATION)

QUESTIONS

1. Discuss critically the method demonstrated?

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2. What conclusions can be drawn about the underline physiological principles involved in the various methods used?

_____

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QUESTIONS

1. What value did you obtain for control blood flow to the forearm?

_____

_____

2. What values of flow were obtained during cooling, warming and after arterial occlussion for 2 minutes?

_____

_____

_____

_____

3. Discuss critically the method demonstrated

_____

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4. What conclusions can be drawn about the underline physiological principles involved in the various methods used?

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EXPERIMENT NO. 13

RECORDING OF HUMAN ELECTROCARDIOGRAM (E.C.G.)
(DEMONSTRATION)

QUESTIONS

1. What are the normal values for the PR interval, QRS duration and ST intervals?

_____

_____

2. What do prolongations of the PR interval and QRS duration denote respectively?

_____

_____

_____

How can you explain differences in the appearance of the QRS complex from V_1 to V_6 in terms of the mean electrical axis of the heart?

_____

_____

_____

What is the normal range for the mean electrical axis of the heart?

_____

_____

_____

_____

What do right and left axis deviations indicate?

_____

_____

_____

Use extra sheet(s) of paper if necessary

SECTION III

RESPIRATION AND METABOLIC RATE

Experiment 14:	Respiratory Function Tests
Experiment 15a	Effects of exercise on cardiovascular and pulmonary systems in Man
Experiment 15b	The effect of oxygen lack and carbon dioxide excess, separately and together, on breathing in man, (Douglas and Presstley's Experiment).
Experiment 16a	Peak Expiration Flow (P.E.F.)
Experiment 16b	Effects of Breath-holding , hyperventillation, etc. on Respiration
Experiment 17	Resting Pulmonary gas Exchange.

EXPERIMENT NO. 14

RESPIRATORY FUNCTION TEST

QUESTIONS

1. From your record, calculate the number of respiration per minute, inspiratory reserve volume, inspiratory capacity, expiratory reserve volume. Correct to body temperature.

_____

_____

_____

2. calculate and record the ratio: $\frac{\text{Vital capacity}}{\text{Surface area (in } M^2\text{)}}$

Compare with normal values in a Handbook/Textbook.

_____

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_____

3. What are the normal ranges for the measurements you have recorded?

_____

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4. determine the percentage of the vital capacity in the first, second and third seconds of the exhalation (times vital capacity).

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5. Calculate the ratio:

$\frac{FEV_1}{FVC} \times 100$

_____

_____

6. Name one condition (apart from bronchial asthma) in which there is an obstructive defect (N.B.: There is greater reduction in FEV_1 than in FVC. The ratio FEV/FVC% may be as low as 40%).

_____

_____

7. Summarize and discuss your findings

_____

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_____

EXPERIMENT NO. 15a

EFFECTS OF EXERCISE ON CARDIOVASCULAR AND PULMONARY SYSTEMS IN MAN

The experiment is designed to measure the respiratory and cardiovascular response to exercise in man. The exercise should take the form of stepping on and off at a constant rate.

The height of stool in meters multiplied by the weight of the body, in kg., gives the work in kg. M/per step, and this multiplied by the number of steps per minutes give the rate of work in kg. M/mls.

Protocol

1. With the subject standing quietly and breathing through the atmosphere, collect the expired air for a period of 3 minutes into one of the Douglas bags. Count the rate respiration, pulse rate and measure the resting blood pressure and the volume of expired gas.

2. Start exercise at a constant rate of 20 – 30 steps/minute, allowing the subject to expire into one of Douglas bags 3 minutes and after 6 minutes.

3. Repeat step 2 after a complete recovery for 5 minutes and take the readings immediately, after 3 minutes and after 6 minutes.

4. Tabulate the results as shown below:

Parameters	**Control**	**Exercise**		**Recovery**	
		3 min.	**6 min.**	**3 min.**	**6 min.**
1. Rate of Respiration					
2. Respiratory minute volume					
3. Heart rate					
Blood pressure					
(a) systolic					
(b) diastolic					

EXPERIMENT NO. 15b

THE EFFECT OF OXYGEN LACK AND CARBON DIOXIDE EXCESS, SEPARATELY AND TOGETHER, ON BREATHING IN MAN, (DOUGLAS AND PRESTLEY'S EXPERIMENT)

APPARATUS: Spirometer with recording drum and soda-line cannister. Airway tubing with valved mouth-piece and noseclip. Oxygen cylinder.

METHOD: Generally, Spirometer is arranged for re-breathing round a circuit. The subject should be used to mouth-piece breathing and sits quietly. The flat tap first connects the subject's airways to atmosphere. When he is quite settled and relaxed, turn the flat tap to connect the subject to the spirometer. Continue re-breathing for 5 min. but stop before then if the subject is distressed or shows signs of cynosis of fading consciousness. he should be warned that he will feel that his breathing is greater than usual.

	Soda-lime cannister	Gas-in spirometer
Hypoxia alone	in expiratory airway removed	room air
Hypercapnia alone	removed	oxygen
Both stimuli together	removed	room air

ANALYSIS/QUESTIONS

1. Over half-minute periods, measure the average tidal volume and the number of breaths. Their product gives a quantitative measure of ventilation.

_____

_____

2. Compare the three traces. What is the ventilation at the end of the shortest trace, or at 5 minutes?

_____

_____

3. Do you think the two stimuli have an additive effect or is there evidence of interaction between them.?

_____

_____

_____

_____

_____

EXPERIMENT NO. 16a
PEAK EXPIRATION FLOW (P.E.F.)

QUESTIONS

1. How does the calculated value compare with your own measured value?

_____
_____
_____

2. Would PEF be of use in the diagnosis of restrictive type of ventillatory defect? Explain your answer.

_____
_____
_____
_____
_____

EXPERIMENT NO. 16b

EFFECTS OF HYPERVENTILATION, BREATH-HOLDING AND RE-BREATHING FROM PLASTIC BAG ON RESPIRATION

A. HYPERVENTILATION

QUESTIONS

1. Desribe the subjective feeling that you experienced after hyperventilation. Can you explain why?

   _____

   _____

   _____

   _____

   _____

2. Explain the respiratory pattern observed after hyperventilation.

   _____

   _____

   _____

   _____

   _____

3. Explain your observations during rebreathing.

   _____

   _____

   _____

   _____

EXPERIMENT 17a

RESTING PULMONARY GAS EXCHANGE

QUESTIONS

1. Compare the composition of samples of inspired (room air) and expired air.

_____

_____

_____

_____

_____

_____

2. What are the major changes due to pulmonary gas exchange?

_____

_____

_____

_____

_____

_____

EXPERIMENT NO. 17b

ANALYSIS OF ALVEOLAR AND EXPIRED AIR

QUESTION

1. Explain the difference between expired air and alveolar air.

_____
_____
_____
_____

SECTION IV (KIDNEY AND BODY FLUID)

Experiment 18: ESTIMATION OF BLOOD VOLUME OF A RAT (DEMONSTRATION)

Experiment 19: HUMAN DIURESIS

Experiment 20: URINALYSIS

Experiment 21: URINE FORMATION

EXPERIMENT NO. 18

ESTIMATION OF BLOOD VOLUME OF A RAT (DEMONSTRATION)

EXPERIMENT NO. 19

HUMAN DIURESIS

QUESTIONS

1. What % of the water or saline load was excreted by each subject during the experimental period. Comment on the observed differences.

_____
_____
_____
_____
_____
_____

2. Do you have evidence to support the view that vasopressin is an anti-diretic hormone? What is its mode of action?

_____
_____
_____
_____
_____
_____

3. Discuss the mechanism involved in the control of water excretion and the mode of action of the various substances used in the experiment.

_____
_____
_____
_____
_____
_____

EXPERIMENT NO. 20

URINALYSIS

QUESTION

Explain the following: Alkalosis, and Acidosis, Benign glucosuria

_____

_____

_____

EXPERIMENT NO. 21

URINE FORMATION IN MAN

ASSIGNMENT OF STUDENT ACTIVITIES IN EACH GROUP

Time subject	A Control water Diuresis	B Saline dieresis	C Effect of Kolanut	D Effect of Previous dehydration
Day before laboratory Period, meal as usual after Fluid as specified P.m. lunch	Water and fluids as usual	Water and fluids as Usual	Water and fluids as usual	No water or drinks 2.

Night before lab. 8.00 p.m	Each subject voids and discard urine			
From 8.00 p.m. night Before, to 8.00a.m. Day of laboratory	After 8.00p.m each subject is to save all urine including final voiding at 8.00a.m. on the day of laboratory. The urine of each subject is pooled to provide a12 hour sample. The volume, specific gravity and urinary osmolality of each pooled samples is measured as specified under procedures. Each subject may breakfast, but no fluids except as specified.			
From 8.00 a.m to 8.20a.m. on day of laboratory	Drink 1200cc of water, 15ml/kg	Drink 1200cc of 0.9% NaCL, 15ml/kg	Drink 1200 of water and take kolanut	Drink 1200cc of water, 15ml/kg.
From 8.30 a.m. to 8.30p.m.	Collect urine at 30 minutes intervals, more often if necessary. On each sample, record time, measure volume, specific gravity, and estimate the osmolality.			

2.30 p.m.	Complete all analysis.
Before leaving laboratory	Enter result in appropriate spaces on large sheet of paper which will reflect individual

Results:

1. If the 12 hr. urine was accurately collected, calculate the rate of urine flow in m1/min. what is the effect of dehydration on urine flow?
   `
2. Construct histograms to show each of the following varies with time: (a) urine flow (b)urinary osmolalities. How do these variables relate to each other?

3. Comment on the differences in response in the groups of Experiments.

This experiment is an example of volume expansion. Which body fluid compartment (s) is affected by drinking (a) water (b) saline

Procedure for Dehydration.

A. Day before Experiment

1. No water or drinks (e.g. bear, spirits, coke, etc.) after lunch
2. Continue to void and discard urine until 8:00 p.m.
3. At precisely 8:00 p.m. void and discard urine
4. After (3) above all urine (including early morning urine) must be kept in the container provided.

.

5. Report at the Laboratory with your container before 8:00 a.m. the next day.
6. Please do not take water or drinks during all this period.
7. As much as possible limit strenuous physical activity.

B. <u>Day of Experiment</u>

1. At 8.00 – 8.15a.m empty bladder into the urine already collected.
2. Therefore we should have precisely 12hours urine sample, and hence 24 hour urine volume can be calculated.
3. At 8.15a.m kola nut is given if you are required to take kola nut, (chew the kola thoroughly and swallow it).
4. Weigh yourself to the nearest 0.1kg.
5. 8.30a.m saline or water load (1,2L) is taken.
6. At 9.00, 9.30, 10.00, i.e. 30 minutes interval empty bladder and separate containers provided.

QUESTION

Describe briefly the clinical import of this experiment.

_____

_____

_____

SECTION V (GASTROINTESTINAL SYSTEM)

Experiment 22: Salivary secretion

Experiment 23: Collection and analysis of gastric juice in rat

Experiment 24: Intestinal motility

EXPERIMENT 22

SALIVARY SECRETION

DEMONSTRATION OF ENZYME ACTIVITY

Record and compare the times taken for this end point in each of the four samples (I, II, III and IV).

Sample	Iodine reaction							
	2min	4min	6min	8min	10min	12min	14min	16min
I								
II								
III								
IV								

N.B. Use the following scoring technique.

* low
** Moderate
*** High

INVESTIGATING THE DIGESTIVE ACTION OF SALIVA

(1) Place the piece of bread provided in your mouth and without chewing it, note its taste and record.

(2) Now chew it for about 5 – 6 seconds and again note and record its taste.

(3) Continue chewing it for as long as you like till it is ready for deglutition and just before you swallow please note and record the taste for a third time. Is there any difference between its taste during the three occasions? You may repeat the entire experiment if you are not so sure you have recorded the actual tastes you sensed. Be precise.

(4) Explain your findings.

_____
_____
_____
_____
_____
_____
_____
_____
_____

Questions:

1. What are the effects of different substances used on salivary secretion?

2. What is the difference between conditioned and unconditioned reflex secretion of saliva?

3. What is the effect of salivary amylase on carbohydrate digestion?

4. What do you understand by spontaneous and paralytic secretion of saliva?

5. What is Aptylism?

6. List the functions of saliva

EXPERIMENT 23

COLLECTION AND ANALYSIS OF GASTRIC JUICE IN RAT

INTERPRETATION OF RESULTS

Express your results in the form of a table and graph. Plot the time (minutes) along the abscissa, mark the scale of pH along the right-hand ordinate and the acid concentration (meq/litre) along the left-hand ordinate

	Time (Min)	Gastric acid Output			Total concentration Corresponding to time interval (meq/L/15min)	Mean secretion at time interval
		Rat 1	Rat 2	Rat 3		
Basal Secretion	15					
	30					
	45					
	60					
Maximal Secretion	75					
	90					
	105					
	120					

EXPERIMENT 24

INTESTINAL MOTILITY

QUESTIONS

1. Describe the movement observed in the isolated rabbit ileum under normal conditions. What is the function of the movement?

2. What is the effect of different drugs used and mechanism of their action?

3. What is the main difference between Ringer, Ringer-locke's and Tyrode solution?

4. Describe the types of movements in the small intestine

5. List the differences between intestinal smooth muscle and skeletal smooth muscle.

_____

_____

Use extra sheets if necesar

SECTION VI - REPRODUCTIVE ENDOCRINOLOGY PRACTICALS

Experiment 25 : A Study of Estrous Cycle in Rat by Vaginal Smear Technique

Experiment 26 : Semen analysis

EXPERIMENT 25

A STUDY OF ESTROUS CYCLE IN RAT BY VAGINAL SMEAR TECHNIQUE

QUESTIONS:

1. What is the significance of taking vaginal smears in human and their clinical relevance?

2. What are different phases of estrous cycle?

3. What do you mean by reflex ovulation?

4. Draw a diagram showing timing of estrous cycle and menstrual cycle.

5. What is the significance of detecting ovulation time?

6. What is safe period?

EXPERIMENT 26

SEMEN ANALYSIS

A. SPERM MOTILITY

QUESTIONS:

1. What are morphological characteristics of the sperm?

_____
_____
_____
_____
_____

2. What factors influence their motility?

_____
_____
_____
_____
_____

3. Mention a few morphological abnormalities of sperm and their effect on fertility?

_____
_____
_____
_____
_____

4. What is normal sperm count per ejaculate?

_____
_____
_____
_____
_____

5. What are the functions of the epididymis?

_____
_____
_____
_____
_____

6. What is the difference between spermatogenesis and spermiogenesis?

_____
_____
_____
_____
_____

7. How does variation in temperature affect spermatogenesis?

_____
_____
_____
_____
_____

8. What happen when the vasa deferential are tied?

_____
_____
_____
_____
_____

SECTION VII

EXPERIMENTS ON THE NERVOUS SYSTEM

INTRODUCTORY NOTES ON NERVE MUSCLE PREPAREATION

EXPERIMENT 27: Simple Isotonic Muscle Twitch and The Effect of Temperature on A Simple Muscle Twitch.

EXPERIMENT 28: All or None Law, refractory Period, Tetanus and Fatigue

EXPERIMENT 29: Velocity of Nerve Conduction. The Effect of Load and length on Muscle

EXPERIMENT 30: To demonstrate an Isometric Contraction in a Nerve-Muscle Preparation

EXPERIMENT 31: Reflex Action In Man

EXPERIMENT 32: Reflex in Spinal Frog

EXPERIMENT 33: Sensation

EXPERIMENT 34: Vision I

EXPERIMENT 35: Vision II

EXPERIMENT 36: Hearing and Balance

EXPERIMENT 37: Reaction Time

EXPERIMENT No. 27

SIMPLE ISOTONIC MUSCLE TWITCH AND THE EFFECT OF TEMPERATURE ON A SIMPLE MUSCLE TWITCH

CALCULATIONS

Using the time trace, calculate the following:

a. latent period (msecs)

_____

What causes this?

_____

_____

_____

_____

_____

b. contraction period (msecs)

_____

c. relaxation period (msecs)

_____

d. Measure the height of contraction (h).

_____

From the following measurements:

a – length of lever (cm)
p – distance of weight from fulcrum (cm)
m – weight applied to lever (g)
b – vertical distance from fulcrum to shread attachment of muscle to lever (cm).

Then

(i) Actual shortening of muscle (x) $= \frac{hb}{a}$ cms

(ii) Mass lifted by muscle (y) $= \frac{pmg}{b}$ dynes

(iii) Work done by muscle (w) $= \frac{pmgh}{a}$ ergs

(B) THE EFFECT OF TEMPERATURE ON A SIMPLE MUSCLE TWITCH

Calculate and tabulate for the three temperature as follows:

Temperature	**Twitch**			
	Latent period	**Contraction period**	**Relaxation period**	**Work done**
Room Temperature				
Warm				
Cold				

QUESTIONS

1. Discuss your results and suggest reasons why contraction height with a simple weighted lever may be an unreliable guide to the effect of temperature on muscle contraction.

_____

_____

_____

_____

_____

_____

_____

2. What has been the effect of temperature on nerve conduction?

_____

_____

_____

_____

_____

EXPERIMENT NO. 28

ALL OR NONE LAW, REFRACTORY PERIOD, TETANUS AND FATIGUE

A. A STUDY OF THE EFFECT OF STIMULUS STRENGTH ON MUSCLE CONTRACTION (ALL OR NONE LAW).

QUESTIONS

1. How does the tracing fit in with the all or none law?

_____
_____
_____
_____
_____
_____

2. Why is the result for skeletal muscle so different to that obtained with cardiac muscle?

_____
_____
_____
_____
_____

3. Discuss the above experiment in relation to motor-unit.

_____
_____
_____
_____
_____

B. EFFECT OF TWO STIMULI IN CLOSE SUCCESSION (ABSOLUTE REFRACTORY PERIOD)

QUESTIONS

1. Describe your observations

_____

_____

_____

_____

_____

2. What is the duration of the refractory period? (i.e. the duration after the application of one stimulus during which a second stimulus has no effect).

_____

_____

_____

_____

_____

C. THE PRODUCTION OF A SUSTAINED CONTRACTION (GENESIS OF TETANUS)

QUESTIONS

(a)…What was the effect on height of contraction?

_____

_____

_____

_____

_____

(b) What effect did fatigue have on latent period?

_____

_____

_____

_____

_____

(c) What effect did fatigue have on the contraction and relaxation phases?

_____

_____

_____

_____

_____

EXPERIMENT NO. 29

VELOCITY OF NERVE CONDUCTION. THE EFFECT OF LOAD AND LENGTH ON MUSCLE

A. VELOCITY OF NERVE-CONDUCTION

A nerve such as the sciatic nerve is made up of many individual nerve fibres, of different

QUESTIONS

1. How does the velocity of conduction vary with temperature?

_____

_____

_____

_____

2. Are the results comparable to what you would expect from your knowledge of nerve conduction in mammalian nerve?

_____

_____

_____

_____

_____

_____

3. What are the factors affecting condition of nerve impulses?

_____

_____

_____

_____

_____

_____

_____

_____

_____

2. THE EFFECT OF LOAD AND LENGTH ON MUSCLE

What to do

1. Calculate the work done at each twitch and draw a graph of work done, W (ordinate) against load, M (abscissa) both for pre and after loading.

Mass (gm)	After loading			Free loading		
	H (cm)	h (cm)	W (g-cm)	H (cm)	h (cm)	W (g-cm)
10						
20						
30						
40						
50						
60						
70						
80						
90						
100						

Attach graph

2. What do you mean by "optimum load?

_____

_____

_____

_____

_____

EXPERIMENT NO. 30

TO DEMONSTRATE AN ISOMETRIC CONTRACTION IN A NERVE-MUSCLE PREPARATION

Tabulate the results as follows:

Muscle length (cm)	Tension in muscle (g. wt.)		Potential energic (1/6 TL (g.cm)	
	Resting (stretched)	Stretched	Resting (stretched)	Stimulated

Where:

The maximum tension developed in the muscle (g.wt.)
L = Length of muscle (cm).

Graph the results to indicate:

1. The work done (or potential energy developed in g/cms) during contraction (as ordinate) relative the tension on the resting muscle (g), as the abscissa.

2. Resting tension against increase in tension during contraction.

Compare the results with those obtained for isometric contractions.

_____
_____
_____
_____

How much of the potential energy of isometric contraction is converted into useful work when the muscle is allowed to shorten (at the point of maximum isometric contraction)?

_____
_____
_____
_____

EXPERIMENT NO. 31

REFLEX ACTION IN MAN

QUESTIONS

1. Discuss your results explaining the various pathways involved.

_____
_____
_____
_____
_____
_____
_____
_____

2. On what part of the nervous pathway would you expect to find an interruption if (1) a tendon reflex was absent; and (2) a tendon reflex was exaggerated? Explain the physiological principles involved.

_____
_____
_____
_____
_____
_____
_____

EXPERIMENT NO. 32

REFLEX IN THE SPINAL FROG

QUESTIONS

1. What are body righting reflexes?

2. What is spinal shock? How are the flaccid paralysis and hyporeflexia accounted for?

3. Define the following
   Withdrawal reflex, irradiation, crossed extensor reflex reciprocal innervations, after discharge summation.

4. What is the mechanism concerned in each of the phenomena?

5. What is titanic contraction? Discuss the mechanism.

EXPERIMENT NO. 33

CUTANEOUS SENSATION

TEMPERATURE

What to do

1. Compare the difference in temperature sensation experienced by holding (a) a piece of cloth and (b) a piece of metal both of which should be at room temperature.

_____
_____
_____
_____
_____
_____
_____

2. Try to explain this on the basis of the physical changes occurring in the skin.

_____
_____
_____
_____
_____
_____
_____

3. PAIN

Localisation of pain spots

This experiment is connected with fast or pricking pain. Very little pressure is required to elicit this pain from uncallused skin.

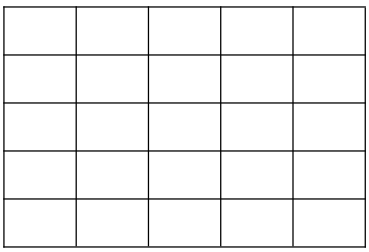
1. Blindfold the subject
2. Using lancet to apply brief, light stimuli, map out sensitive areas within the grids used for mapping touch spots.
3. Check for adaptation by continuously applying painful stimulus for 15 seconds and 2 minutes.

NOTE: The subject should report when the stimulus elicits a sensation of pain. NOT WHEN the stimulus can be detected by its pressure.
OBSERVATIONS

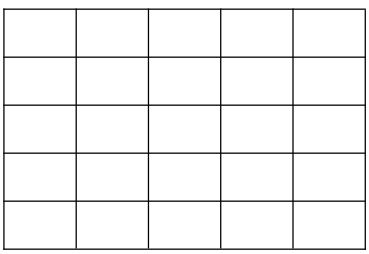
	CODE
Touch	•
Cold	o
Warmth	
Pain	∇

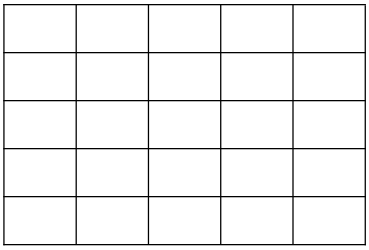
In case of insensitive point put (-) on top of the relevant code.

PALM



FOREARM





QUESTIONS

1. In which region is sensitivity of spots greatest for touch, pain and temperature? What determines this?

_____
_____
_____

Does two point discrimination vary with the region?

_____
_____
_____

2. What is the relationship between two point discrimination and density of sensitive spots?

_____
_____
_____
_____
_____

3. Does error of localisation vary with the region?

_____
_____
_____
_____
_____

4. Is the ability to localise related to density of spots in that region? If so how?

_____
_____
_____
_____
_____
_____

5. According to the results of experiments, give the sequence of adaptation of different sensations

_____

_____

_____

_____

_____

6. Does the thermal sensation indicate absolute temperature or relative temperature?

_____

_____

_____

_____

_____

7. Is there any other aspect of thermal stimulus involved in thermal sensation?

_____

_____

_____

_____

_____

8. Are there pain spots and analgesic areas of skin-tested? Do pain, cold, warm and spots coincide?

_____

_____

_____

_____

_____

EXPERIMENT NO. 34

VISION 1

A. Visual Acuity

Investigate the effect of putting lenses (concave and convex) in front of the eye. Describe the effect observed, particularly in cases of myopia and hypermetropia.

_____
_____
_____
_____
_____
_____
_____
_____

B. BLIND SPOT

Approximately how many times the diameter of the blind spot is this?

_____
_____
_____
_____
_____

D. NEAR POINT

What influence does age have on the near point.

_____
_____
_____
_____
_____

E. FOCUSING

F. SCHEINER'S EXPERIMENT

Draw diagrams for each case to show the paths of rays from the two objects to the retina.

Space for diagram

Space for diagram

G. RETINAL VESSELS

H. STEREOSCOPIC VISION

Note if there is any difference in the ease with which the experiment is completed and one eye alone is used if the hand holding the thread and the hand holding the needle approach one another (a) along the visual and (b) at right.

_____
_____
_____
_____
_____

EXPERIMENT NO. 36

HEARING AND BALANCE

A. HEARING

What to do

Plot a graph of hearing loss against frequency using a different symbol for ear.

B. BALANCE (VESTIBULAR FUNCTION)

Which of the canals has been affected by these tests?

_____

_____

_____

"Repeat the rotation with the subject's head bent forward" at an angle of 120° - the head can return to the vertical when the rotation has ceased. Compare the nystagmus with the observer above. Repeat the rotation again with the head bent forward on the left shoulder. Spin anticlockwise, then repeat the experiment – again with the head on the left shoulder spinning the chair clockwise.

In each case, describe the nystagmus produced, name the semi-circular canals affected, and indicate the nerve pathways involved.

_____

_____

_____

_____

_____

_____

_____

_____

b. Barany's Test

QUESTION

Give a full account of your results and of the physiological principles involved.

EXPERIMENT NO. 37

REACTION TIME

(c) Auditory Reaction Time

The operator connects the switch to terminal 'S'. Put the marker on to the drum. When the operator depresses button 'O' the bell will sound. The observer depresses the switch opposite the bell to release the marker as in previous experiments.
Measure the auditory reaction times in three experiments and see if improvement occurs.

What to do

Tabulate the reaction times with the various stimuli and the result of practice.

QUESTION

Discuss the various factors involved in producing a time delay between the application of a stimulus and the motor response.

_____

_____

_____

SCORE SHEET

Experiment/Date	Demonstrator's short remark\comment	Score (Max. of 10)

Appendix I (b)

(Appendix to Experiment 6(b))

Choice of ESR Method

The ESR can be carried out by using the Westergren or the Wintrobe method. Using the Westergren pipette is more accurate and is therefore recommended, but both methods are described as a laboratory may have only equipment for the wintrobe method.

The Westergren Method

Required: Westergren pipette and stand.

A westergren pipette has a diameter of 2.5 mm, and is graduated in millimetres from 0 – 200 mm. The graduations must be read carefully as there are different ways of making the pipette. There are different types of Westergren stand. It is important that the stand is perfectly level on the bench and it is usually fitted with a spirit level for this. The stand must not be placed in direct sunlight, because an increase in temperature will increase the rate of sedimentation.

Pipette 0.4ml of 3.6 percent sodium citrate into small containers, previously marked to hold 2 ml. The anticoagulants in the small containers can be kept for several months, if stored in a cool place.

Method

1. Using the small previously prepared container, add 1.6 ml of venous blood to 0.4 ml of sodium citrate anticoagulant and mix well. Blood which has been taken into sequestrene can also be used. 1.6 ml of the sequestrenated blood is marked with 0.4 ml of sodium citrate.

2. Using the Westergren pipette, draw the well-mixed blood up to the 0 mark, avoiding air bubbles. The test should be set up as soon as possible after the blood is taken, because the cells swell when left in the sodium citrate for longer than about 2 hours, making the ESR unreliable.

3. Place the pipette upright in the stand, and leave undisturbed for 1 hour.

4. Read in mm the level at which the plasma meets the red cells.

Normal Values

The normal values in tropical countries differ slightly from those in temperature countries. For tropical countries they are as follows:

Men	1 – 10 mm/hour
Women	3 – 14 mm/hour.

It is important to check a very low ESR, to make sure that the blood has not clotted in the tube. This can happen if it is not well mixed with the anticoagulant at the time it is taken.

The Wintrobe Method

Required: Wintrobe haematocrit is a narrow tube with two sets of graduations:
0 – 100 mm for reading the ESR
100 – 0 mm for reading the packed cell volume.

The wintrobe stand, like that of the Westergren, is fitted with a spirit level to make sure that it is level on the bench. It too must not be placed in direct sunlight.

Method:

1. Blood should be collected with proper anticoagulant in proportion to volume of blood to avoid shrinkage of erythrocytes.

EDTA (1 – 2 mg/ml blood) is suggested.

2. Thoroughly mix the blood with anticoagulant immediately before filling tube.

3. Use a filling needle, Plasteur pipette or other cannula that will reach the bottom of tube. Slowly fill the tube with blood avoiding air bubbles in column.

4. Adjust meniscus of specimen to 0 line at top of tube.

5. Place tube in upright position in rack that will maintain vertical position.

6. At end of one hour (and at intervals before that time, if desired) read fall of erythrocytes by recording level of erythrocytes in tube. Erythrocyte sedimentation rate is read on same side of tube as line used in filling and is recorded as mm fall of cells in one hour.

7. At the end of one hour, centrifuge sedimentation tube at 2000 rpm for 15 – 30 min. (if sedimentation rate is not determined, tube may be centrifuged immediately after filing. Steps 1 – 4).

8. Record macrohematocrit as level of packed cell volume of erythrocytes read from scale on tube with 0 line at bottom.

Normal values

The normal values for the Wintrobe method are different from those of the Westergren because, in each, different equipment and anticoagulants are used. The following values are for temperate countries. Those in tropical countries may differ slightly.

Men	2 – 9 mm/hour
Women	2 – 15 mm/hour.

Appendix 1 c (Appendix to experiment 6a)

Blood Groups

a. ABO

There are many systems for grouping blood, but the most common method is probably the ABO system. A subject's blood is categorised according to the presence (or absence) in the blood of agglutinogens (an antigen) which, if present, are attached to the R.B.C's membrane. If a particular agglutinogen is present then there is no comparable agglutinin present in the plasma (see Table). Mixing of incompatible bloods results in agglutination of the RBC's.

BLOOD GROUP	AGGLUTINOGEN (RBC)	AGGLUTININ (Plasma)
0	-	A & B
A	A	B
B	B	A
AB	AB	-

This principle is used in testing for ABO grouping. A blood sample is treated with both anti-A and anti-B anti-sera. From the agglutination pattern resulting in the RBC's, the blood group can be determined. Anti-A and Anti-B sera are provided.
Determine your own blood group.

(b) Rhesus grouping

About 1940 Landsteiner and Wiener injected cells from a Rhesus monkey into rabbits and guinea pigs which subsequently produced anti-bodies against the Rhesus monkey cells. This anti-body was shown to agglutinate red cells in about 85% of humans. This value may be different in black races. Landsteiner and Wiener suggested that this serum was detecting a previously underscribed antigen, which may or may not be presently on human red cells; they called this antigen 'Rhesus'. The discovery in 1941 by Levine and his colleagues that the anti-bodies against this antigen could cause agglutination established its importance clinically. It became apparent later that the reagent produced by rabbits did not.

1. Place 1 drop of test serum on a slide
2. Add 2 drops of blood
3. Mix and spread-evenly over most of the area of the slide
4. Incubate in the oven (pre-set at 39°C) for 2 min
5. Check under the microscope for the presence or absence of agglutination if agglutination is present, then the test is positive and the subject is Rh+.

QUESTION

What is the particular occasion when Rhesus incompatibility can be a problem?
Enter the results of all your determinations, together with answers to questions on the attached sheets.

Appendix 1 d
(Appendix to Experiments on blood)

(C) VINEPUNCTURE

Every doctor **MUST** know the correct technique for venepuncture, but skill in its performance may take long to acquire. You will be taught the correct technique, and the operation will be demonstrated to you. You are advised to practice this skill on a willing colleague **UNDER SUPERVISION**.

Unless there are strong reasons for changing the site for venepuncture, choose a vein on the anterior aspect of the upper third or half of the forearm. The cubital fossa itself should be avoided. A vein may be identified by its colour or by palpation of the site. Identifications becomes easier after the application of a vein tourniquet.

The tourniquet used may be a blood-pressure cuff or a rubber tube. But whatever tourniquet is used, it is important to realise, that the **ONLY** object of applying it for venepuncture is to block the venous return from the forearm. Apply the tourniquet to the upper arm just tightly enough to do this.

Choose a clean, sterile, sharp needle of size 22. Fix it to a 10 ml syringe.

The subject should be seated comfortably on a chair or lying down in bed with his forearm **FULLY** extended. See and palpate the veins that stand out after application of the tourniquet. A vein support on either side by subcutaneous fat is most suitable for venepuncture. Identification of such a vein requires experience. Indeed, in many patients, such veins may not be readily available.

The skin over the selected vein is cleaned with soap and water if necessary. It is then cleaned with ether, followed by alcohol. The vein is steadied by stretching the skin over it. With the plunger pushed into the barrel as far as it will go, the syringe is held firmly and the needle is then pushed through the skin with the bevel of the needle facing upwards. The needle is then rotated slightly so that the vein is entered from the side.

usually, as soon as the needle enters a vein, blood will appear at the nozzle. In collecting venous blood by this technique, sometimes blood will not enter the barrel of the syringe even after the needle has entered a vein. The fault lies commonly in a blocked needle or syringe nozzle, a leak between needle and syringe nozzle, a piston that is too tightly fitting in the barrel or collapsed vein.

But before you withdraw the needle from the vein to repeat the venepuncture, carry out the following simple procedure.

(a) Pull on the plunger very gently

(b) Ask the subject to gently flex and extended fingers of that hand

(c) Gently massage the sides of the forearm between your forefinger and thumb.

QUESTION:

How will (b) and (c) increase the filling of the vein that your needle has entered?

APPENDIX II

17C BREATHING THROUGH TUBES

Introduction

In this exercise, you are to make observation upon the alterations in mechanics of breathing when a subject breathes through:

Experiment (1): a narrow tube: tubes A & E
Or Experiment (2): a large volume tube: tubes C and D
You are expected to do only one of the experiments but you are free to do the other if there is time. For each experiment, a pair of tubes is provided so that you can make comparisons.

Calibration of the tubes:

Experiment (1): Tubes A and B: Take a maximum breath in and blow it out fully through the tube as fast as possible. Time with a stop watch how long it takes, as a measure of the resistance of the tube. Take the average of three values for each tube.

Tube A 1)
2) Average
3)

Tube B 1)
2) Average
3)

Experimental observations:

You are now to observe the effect of breathing through these tubes. Choose one male student as the subject. Observe his breathing.

1. With no tube (i.e. baseline) for 3 minutes
2. Breathing through the tube (i.e. experimental period) for as long as he can without severe distress (when he should remove the tube). Make sure there are no leaks at his mouth. Use a nose clip to prevent breathing through the nose.

Observations: Practice before you start proper observations
The team should work in pairs, one person observing each parameter and one person observing writing down. Use stop watches.

Pair No. 1: Rate of breathing (breathes/Min.): Count the number of breath every 15 seconds, later multiply by 4.

Pair No. 2: Depth of breathing: This is estimated subjectivity by the hands placed on the lower ribs. Score as:

Resting:

1+

2+

the subject to make a maximum deep breath.

Pair No. 3: Use of accessory muscles: Time the appearance of the use of sternomastoids: and (for Experiment 2), time of appearance of warm air at the end of the tube. This will occur at the end of expiration, but not at first – what does it represent?

Finally: The subjects symptoms: How did he feel?
What did he feel made his breathing change?
If he stopped, what made him stop?

After each experiment: note:

Note: The subject should be relaxed as possible and observations should be done quietly and with a minimum of noise and fuss. Do the experiment three times with each tube.
Leave several minutes between observations for the subject to recover
Plot your results against time.

Results: Write the methods briefly and plot your results. make notes explaining the following:

1. What alteration in the mechanics of breathing was imposed by the tubes?
2. What alterations in breathing occurred – and why?
3. Offer an explanation of why the subject had to stop breathing through the tube.

17D….RECORDING CHEST MOVEMENTS DURING RESPIRATION

Since the lungs expand during inspiration and collapse passively during expiration with the thoracic cage movements, various forms of apparatus are in use to register the excursion of the chest wall during respiration. The stethograph, used for this purpose is made up of corrugated rubber hose-pipe wound round the chest.

Apparatus and Material:

Strethograph
Recording
T-tune
Marey's tambour
Stop Watch.

Procedure:

1. Tie the stethograph round the chest wall of the subject at the level of the nipples.

2. Adjust the pressure within the Marey's tambour connected to the stethograph so that the lever is placed horizontally. This is done through the T-tube.

3…During quiet respiration, record 20 respiratory movements (slow drum); three on a faster drum to show the peculiarities of the Curve. record the time in seconds in both cases. From the tracings, determine the following data:

a. The rate of respiration per minute

b. The relative duration of inspiration and expiration

c. The pause between inspiration and expiration; and that between expiration and inspiration

d. The relative speed of inspiration at the beginning and at the end of the phase; and that of expiration.

4. Record the respiratory movements through a control phase and then record the movements while performing each of the following acts:

a. Drinking Water/Swallowing

b. Reading aloud for say 15 sec.

c. Coughing.

d. Holding your breath for as long as possible at the end of a normal inspiration and recording the respiration that follows this period of *Voluntary apnea*

e. respire rapidly and forcibly for two minutes or less. You should only force the inspiration, but allow the expiration to occur passively.

5. Write a report on the experiment and comment on your results.

6. **NB** (i) The stethograph should not be too tight or too loose on the chest

(ii) The subject should not see his tracings while recording. Give him a book to read so as to keep his mind off the experiment. This allows the respiration to take place "normally".

APPENDIX III

(Appendix to experiment 31, pp. 134).

Reflexes in Man

Name of Reflex	Method of Eliciting	Afferent Nerve	Center	Efferent Nerve	Effect
Corneal	Touching cornea	Cranial	Pons	Cranial VII	Eyelid closes
Palatal	Touching soft palate	Cranial IX	Medulla	Cranial X	Palate is elevated
Upper Abdominal	Stroking abdomen	T7 to T10	T7 to T10	T7 to T10	Drawing in of abdomen
Lower Abdominal	Stroking abdomen	T10 to T12	T10 to T12	T10 to T12	Drawing in of abdomen
Plantar	Stroking sole of foot	Tibial	S1 to S2	Tibial	Plantar flexion of toes
Jaw Jerk	Stroking of shin	Cranial V	Pons	Cranial V	Closure of jaw
Biceps	Stroking biceps tendon	Musculocutaneus	C5 to C6	Musculocutaneus	Contraction of biceps
Knee Jerk	Stroking patellar tendon	Femoral	L2 to L4	Femoral	Extension of Knee
Achilles	Stroking Achilles tendon	Tibial	S1 to S2	Tibial	Extension of foot
Light	Light on retina	Cranial	Midbrain	Cranial III	Pupillary constriction
Oculo-Cardiac	Pressure on eyeball	Cranial	Medulla	Cranial X	Slows heart rate
Cillio-spinal	Pain	Sensory	T1 to T2	cervical sympathetic	Pupillary dilation
Carotid sinus	pressure on carotid sinus area	Cranial IX	Medulla	Cranial X	Slows heart rate and lowers blood pressure.

(Appendix to Experiment 33, pp. 139).

Examination of the Sensory System

The objective of this exercise is to test by the commonly used clinical procedures some normal sensory functions. These tests should be performed on your partner; and they are preferably performed whilst the subjects eyes are closed or the parts examined are screened from his sight.

A. (a) Tactile sensation and Tactile discrimination (two-point-sensation)

(i) With a small wisp of cotton wool (or brush) lightly touch the uncovered surface of the skin. Ask the subject to say 'now' as soon as he feels a tactile sensation. Compare the threshold of sensation on corresponding areas on the two sides of the body. Compare also the threshold of sensation on regions of the skin not covered by hairs with those which are covered. Which areas are more sensitive and why? Also ask the subject to describe the spots where he felt the sensation (power of localization).

(ii) Apply simultaneously the two points of a compass or 2 pencils to the skin of the forearm and determine the least distance between the two points that gives a two point sensation. Repeat the procedure, applying the two points to different parts of the body (tips of fingers, the back, the cheek, the abdomen, the arms and compare the different thresholds obtained). Where is the lowest?

(Appendix to Experiment 33, pp. 140).

Pain Sensation

Prick the skin lightly with a sharp pin. Compare the degree of pricking required to elicit sensation at different parts of the body. Apply deep pressure in pinching the muscles of the forearm and notice the deep pain sensation produced.

Exercise:

(i) What theories can be used to explain the sensation of pain?

(ii) Will cutting the kidneys, lungs, cause pain in a conscious man?

(iii) What is referred to as pain?

Stereognosis:

Put different *familiar* objects in the hand of your subject while his eyes are closed. Ask him to identify them. It is preferable to use objects of similar shape but varying sizes as pieces of coins.

Taste Sensation:

The following solutions are provided:

2. Strong solution of sugar (sweet)
3. Strong solution of salt, NaCl (salty)
4. Weak solution of Citric acid (sour)
5. Weak solution of Quinine (bitter)

Ask the subject to put out his tongue. With a glass rod, apply a small drop of each solution (only ONE at a time) to the tip, the upper surface, the back and the sides of the tongue. Ask the subject to describe where he feels the taste of each solution best. Rinse your mouth after each test. USE THE BITTER SOLUTION LAST. Repeat the test if need be. Draw a map for the tongue showing the taste localization areas, explaining your results.

Exercise

(i) What is dysautonomia?

(ii) How does temperature affect taste sensations?

(iii) Draw the neutral pathway of taste.

(Appendix to Experiment 36, pp. 151)

Hearing

1. Auditory acuity:
   The determination of auditory acuity is ordinarily a complex procedure, but a very simple test can be performed with a ticking watch or a clock.

   (a) Work in pairs for this exercise. The background noise in the room should be minimal.
   (b) Plug one ear and have your partner hold the ticking watch in front of the open ear. As the watch is moved away from your ear, listen carefully and indicate when you can no longer hear the ticking. Your partner should measure and record this distance. Repeat the measurement moving the clock from outside towards the ear until he first hears the ticking. Measure the distance. Is the distance the same and when the watch is moving away?