KOMAR UNIVERSITY OF SCIENCE AND TECHNOLOGY (KUST)

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

HUMAN PHYSIOLOGY (MLS 2415C) LABORATORY MANUAL

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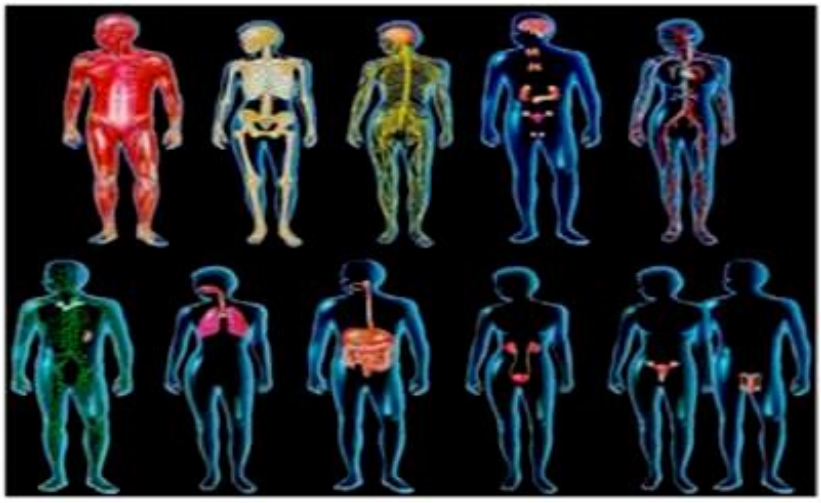
SPRING 2016



Preface

This "Handout" laboratory manual has been prepared mainly in light of two excellent manuals namely Allen−Harper: Laboratory Manual for Anatomy and Physiology, Third Edition, 2010 and Richard Pflanzer: Experimental and applied Physiology, Eighth Edition, 2008. Some modifications has been applied based on the direction and requirements of the Medical Laboratory Science (MLS) department curriculum and study program.

This manual composed of 11 exercises in total focused on the basic concepts of physiology and assists students to get necessary knowledge, skills, and practice in this field. In addition, students will learn how to work safely in physiology labs. Each exercise composed of a short background about a specific topic followed by the principle, Aims, required material, and procedures needed to perform that exercise. The results of each exercise will be recorded by the students and together with the answers of some review questions will be submitted to the lab instructor in the form of lab reports.

Exercises are written in a way to be easy to follow yet informative to the students. The overall laboratory experience reinforces the concepts of the theoretical lectures and together provide a comprehensive knowledge to the students in the field of physiology.

Dr. Heshu Sulaiman Rahman
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Course objectives

-This course starts with an introduction of what science is, and how knowledge is gained by using the scientific method.

-After participating in the course, students would practice safe procedures within a laboratory.

-Students will understand and demonstrate the anatomical position.

-The lab portion of this course provides a solid background to characterize different tissue types.

-Effectively interpret the diagnostic laboratory tests.

-Identify and utilize appropriate reference resources to clarify and expand knowledge pathophysiology.

-This course will provide students with a solid foundation in the fundamental concepts and knowledge base of Human Physiology and related lab experiments.

-This course provides a coherent framework for understanding Human Physiology and prepares students for their upper-level courses.

Rules for laboratory experiments

Experiments done in the laboratories should comply with the existing laws and regulations that are based on bioethical principles.

Basic regulations during lab work

- It is forbidden to touch electric equipment or sockets with a wet hand.
- A functional error has to be announced immediately to the head of the practical.
- Equipments should be only used as described in the lab notes or explained by the supervisor. Any other operation, turning of knobs, flipping of switches, etc. should be avoided as it might lead to the malfunctioning or impairment of the equipment.
- All the electric sockets are situated on the laboratory benches. Protect them from the fluid. Any spillage should be mopped up immediately.
- Accidents which occur in spite of all the preventive measures should be reported immediately to the head of the demonstration.

Biohazards and protection of health

Physiological experiments are made on living animals. A non-anesthetized animal can only be handled by students in the presence of a technician or the instructorof the practical session.

Working with living animals implies the risk of infection, as well. Therefore:

- Eating, drinking, smoking, and chewing are not permitted in the laboratory, except when dictated by experimental protocol.
- Follow the directions of the laboratory book and your laboratory instructor regarding experimental precautions and procedures. Do not deviate without permission. If uncertain or unclear, ask for clarification.
- Use disinfectant to clean laboratory work surfaces before and after procedures.

- Whenever feasible, wear disposable hypoallergenic examination gloves when working with chemicals, body fluids, and animal tissues.
- Eye protection is required for all labs that use chemicals, biologicals, or physically hazardous materials. Safety goggles are preferred. Safety glasses are acceptable, but standard glasses containing corrective lenses are not. Students are responsible for corrective lenses are not.

- In experiments dealing with body fluid (blood, saliva, urine) handle only your own to avoid contamination and transmission of disease.
- Disposable mouthpieces, blood lancets, microhematocrit tubes, and other clean disposable supplies are to be used only once and discarded into appropriately marked containers for disposal. If an experimental procedure must be repeated, use new clean or sterile supplies.
- Nondisposable supplies (test tubes, hemacytometers, etc.) that come into contact with body fluids must be thoroughly cleaned and sterilized after use. Follow the directions of your laboratory instructor.

- Report any accidents, spills, or damaged equipment to your instructor immediately.
- Animal remains (e.g., frog skin, muscles, organs) must be discarded into appropriately marked containers for proper disposal. Wastebaskets and sinks are not appropriate containers for animal remains.

- At the conclusion of a laboratory period, restore your laboratory table to a clean, orderly condition. Wipe the table clean by using napkins and disinfectant solution, clean and return equipment and supplies to their proper place, ensure that the sink is clean and free of debris, and place your chair beneath the laboratory bench.

- Wash your hands thoroughly before leaving the laboratory.
- Violation of laboratory rules and regulations may result in your

being asked to leave the laboratory for your own benefit and safety as well as for the benefit and safety of your classmates. Repeat violators will be dismissed from the class and awarded a failing grade for the semester.

Laboratory safety signs/labels

Laboratory reports

Your write-up for **Human Physiology Lab (MLS2415C)** should be a clear and concise report of the **purpose of the experiments** [1] you did, the way in which they were performed (**Materials and Methods** [2]), the resulting data (**Results** [3]), and your **conclusions** [4] based on these data.

A section of **LABORATORY REPORT QUESTIONS** is located at the end of each exercise. These questions can be answered by the student and handed in for grading at the discretion of the instructor. Even if the instructor does not require you to answer these questions, we recommend that you do so anyway to check your understanding and to use them in your LABORATORY REPORT.

Do not plagiarize when writing Laboratory Reports. Therefore, it is strongly recommended to write your report in your own words.

Title Page

Title of the Experiment

Date

Your Name

Parts of the Laboratory Report

1. Objectives
2. Materials and Methods
3. Results
4. Conclusions
5. References

Experiment 1: Sample collection and preservation

1.1. Blood collection in human

1.1.1. Aims

-To obtain blood for diagnostic purposes.

-To monitor levels of blood components.

-To administer therapeutic treatments including medications, nutrition, or chemotherapy.

- To remove blood due to excess levels of iron or erythrocytes (red blood cells).

-To collect blood for later uses, mainly transfusion either in the donor or in another person.

1.1.2. Background

Blood collection is one of the most important diagnostic tools available to clinicians within healthcare. Its data is relied upon in the clinical setting for interpretation of a myriad of clinical signs and symptoms and developing skills in venepuncture can facilitate holistic and timely treatment.

1.1.3. Principles

Blood handling policy

a. Due to dangerous blood-transmitted communicable diseases, blood samples from unknown donors such as hospital laboratories are not acceptable.

b. However, fresh sampling from the students themselves can still be a safe educational procedure.

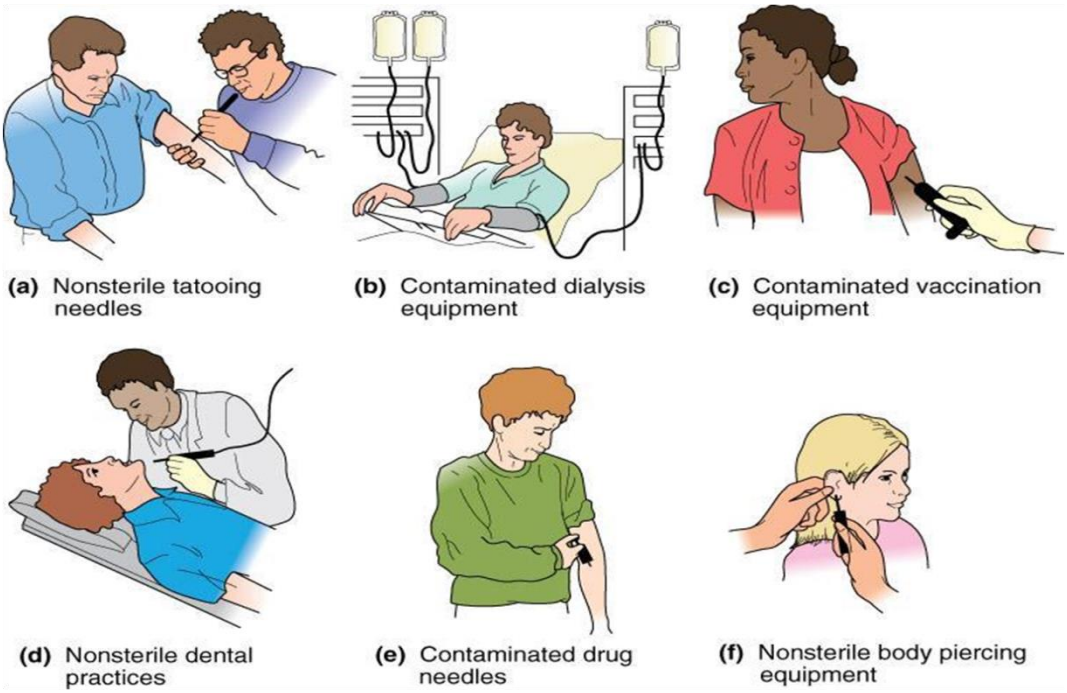
c. Do not participate if you have hepatitis, its antigen, or HIV infection.

d. Do not participate if you have any type of blood clotting insufficiency such as hemophilia.

Sources of infection with blood transmitted diseases

1. Direct contact of contaminated body fluids, mucous membrane, or injured skin.

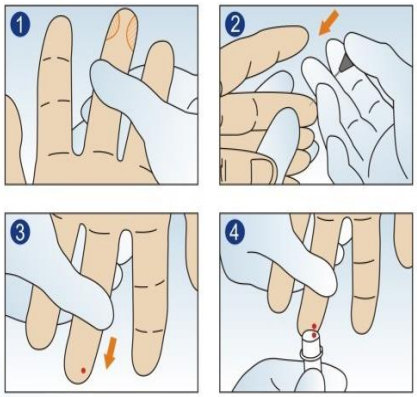
2. Needle stick of skin or use of contaminated instrument to the skin or mucous membrane.



Example of blood transmitted diseases

Causes of
Hepatitis B

1.1.4. Materials and Methods



1.1.4.1. Procedure of blood collection in human

1. Wash hands with soap and water before and after the procedure.

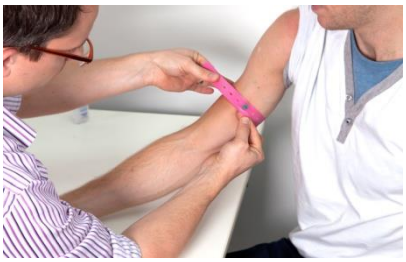
2. Clean fingers with alcohol swab and air dry.

3. The recommended site for capillary collection for adults is the palmer surface of the distal (end) segment of the third (middle) or fourth (ring) finger, ideally of the non-dominant hand. Use lancet or auto-lancet only once.

4. The recommended site for collection of blood from a vein is a radial or cephalic vein. But you need to use tourniquet before venipuncture.

5. Blood should flow smoothly with a minimum of vacuum, and if a syringe is used, one should avoid pumping the syringe barrel.

6. If an unclotted sample is required, the blood is discharged into a test tube with a selected anticoagulant.



7. Adequate mixing is ensured by inverting the tube several times, or by rolling between palms of the hand.

8. Five milliliter blood is sufficient for most hematologic examinations.

1.1.4.2. Precautions

1. Discard any items contaminated with blood in the container such as lancets, glass slides, cover slips, and capillary tubes (do not leave them on the bench).

2. Avoid getting the blood into the mucous membranes of your eyes or mouth or on an area of irritated or damaged skin.

3. Select a site that is warm, pink and free of any cuts, scars or rashes. Avoid skin areas that have evidence of previous punctures.

4. If you do get blood from another person, do not panic; just obtain adequate soap and water and wash immediately and thoroughly (3-minute handwash is recommended).

5. In cases of blood spills by accident or injury, the person should attempt tocleanup, should obtain gloves, place absorbent material over the spill, and flood the area with an approved disinfectant.

1.2. Laboratory animals

1.2.1. Aims

1. To induce some diseases such as cancer, diabetes, hypercholesteraemia, and gastric ulcer.

2. To determine the effectiveness of some compounds and drugs on those diseases.

3. To determine the toxicity of some natural products and chemicals on various tissues and organs.

1.2.2. Background

Most common laboratory animals

1. Mouse

2. Rat

3. Guinea pig

4. Hamster

5. Rabbit

Animal handling and management

-The animals should be kept in polystyrene cages in appropriate number.

- The animals should be acclimatized to the laboratory environment at 24 ± 1˚C under a 12 h dark-light cycle for at least 5 days, before commencement of any experiment.

-The animals should be provided with special pellet and water during the decided period of study.

-The animals were deprived of feed 12 h prior treatments. Oral intubation was applied using a ball-tipped stainless steel gavage needle attached to a syringe.

-The animalsshould beobservedfor clinical abnormalities twice each day (morning and afternoon) fortheonsetofclinicalortoxicological symptoms.

-Mortality,ifany, signs of toxicity, body weight, food consumption and gross findingsshould beobserved overaperiod oftreatment.

-The study should be approved by the Animal Care and Use Committee (ACUC).

-After which the animals should be euthanised, using overdose (usually 0.5 ml) xylazine and ketamine mixture.

-The animal should be immediately excised and vital organs should be collected directly for examination, while blood should be taken for hemogram and blood biochemical analyses.

1.2.3. Materials and Methods

Sample collection in laboratory animals

A. Blood collection

1. Local anesthesia is necessary, especially when the animal is nervous, to control the animal and safe blood sampling.

-Usually intraperitonal injection of a mixture of ketamine-HCl and xylazine (0.2 ml) is used.

2. The best areas of blood sample collection area:

a. Cardiac puncture (the most common site).

b. Caudal vena cava.

c. Retro orbital vein.

3. The precautions and main steps are the same as mentioned

above for human.

B. Organ collection

-Generally vital organs are collected such as liver, kidneys, heart, spleen, lungs and brain.

-The samples should be washed with sterile phosphate buffer solution or normal saline.

-Samples should keep in a plastic container that contained 10% formalin for histopathology and immunoperoxidase examinations or 4% glutaraldehyde for electron microscopy examination.

1.3. Results

-Have you tried to collect blood sample by yourself?

- Were you successful in collecting blood sample?

-Have you tried to handle lab animals?

-Have you sacrificed lab animal? Which anaesthesia you were used? How much the dose?

1.4. Review question

- What are precautions during blood collection in individual that recently has been diagnosed with swine influenza?

-Is there any Zoonotic disease that transmitted from lab animals to human? Explain.

- What are the most common nosocomial disease in the hospitals that may be transmitted from infected human to normal individuals throught direct or indirect contact with blood?

Experiment 2: Osmosis across plasma membrane

2.1. Aims

Predict the effect of hypotonic, hypertonic and isotonic solution on a cell, because regulating the water flow through the plasma membrane is an important factor in maintaining homeostasis within a cell.

2.2. Background

Water diffuses from an area of higher water concentration (lower solute concentration) to an area of lower water concentration (higher solute concentration).

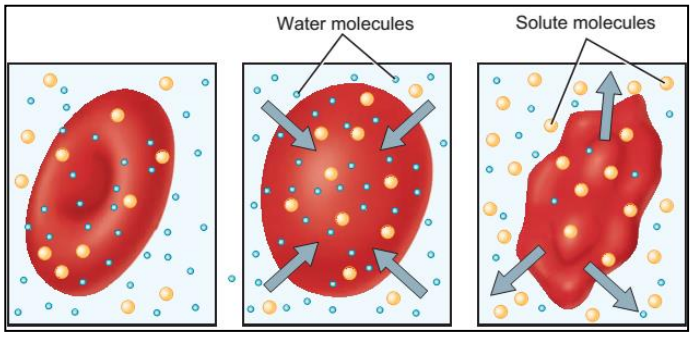
Terms related to the experiment

1. Solvent

It is a dissolving medium, which is water in living organisms.

2. Solute

It is a substance that dissolved in a solvent.



3. Hypotonic

It is a solution with a low solute concentration (high water concentration).

4. Hypertonic

It is a solution with a high solute concentration (low water concentration).

5. Isotonic

It is a solution with a salt (NaCl) concentration of about 0.9%.

6. Diffusion

It is the movement of particles from an area of higher concentration to an area of lower concentration.

Osmosis

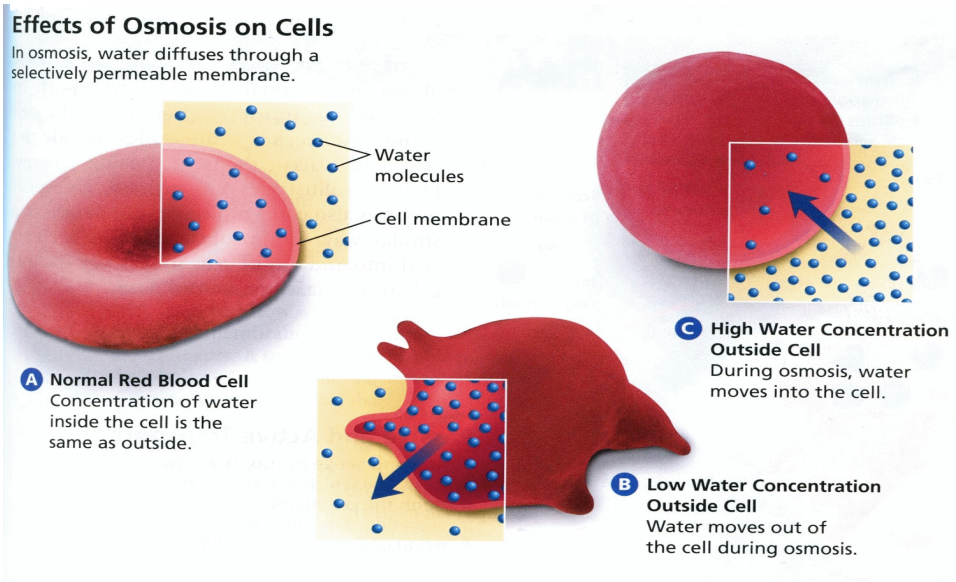
-It is the diffusion of a water (solvent) across a semipermeable membrane that due to differences in solute concentrations.

-In a cell water always move to reach an equal concentration on both sides of the membrane.

-Osmosis results in movement of water from a solution with a lower solute concentration (hypotonic) into a solution with a higher solute concentration (hypertonic).

-Water will move into the hypertonic solution until the solute concentrations of the two solutions equalize to become isotonic solutions (iso-: same).

-Red blood cells (RBCs) are good examples to use for this osmosis experiment because their shape changes dramatically when they are exposed to hypotonic or hypertonic solutions.

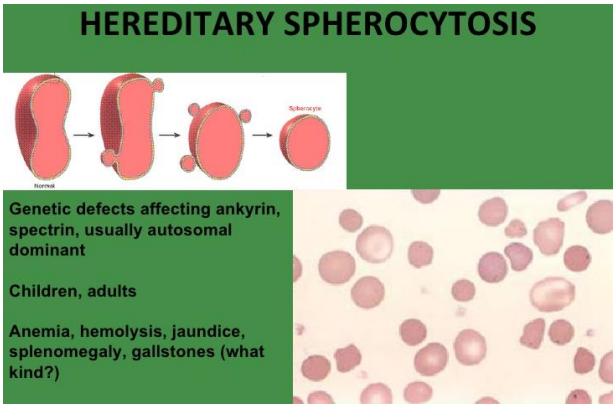


-Their normal shape is around biconcave disc with a smooth plasma membrane. The plasma membrane does not allow salts in the RBC cytoplasm to leave the cell, but water can freely enter or leave the cell through the plasma membrane.

-Under the normal light microscope, RBCs look two-toned, with the center being lighter because of its concavity and thinness.

-If the cell loses most of its water by osmosis when put in a hypertonic solution, it becomes created or shriveled with spiked edges.

-If the cell gains a significant amount of waterby being placed in a hypotonic solution, it swells and may eventually burst—a process called hemolysis (hemo-: blood; -lysis: breakdown).



-As a basis for comparing various solutions to blood, the salt content (NaCl) of blood is 0.9% and is the same salt content as a physiologic salinesolution.

-Hereditary spherocytosis is a common disorder in which red blood cells are defective because of their ball-like (spherical) shape. These cells are more fragile than normal.

-Spherical cells have increased osmotic fragility because they are less likely to expand and break in salter water than normal red blood cells.

2.3. Procedure

2.3.1. Blood collection

-Clean the palmar surface of a finger (third or fourth) with a sterile gauze pad soaked with 70% alcohol or a sterile disposable alcohol prep.

-Allow the skin to dry; do not blow on it to make it dry faster.

-Use a sterile lancet, to make a quick stab wound through the cleansed surface of the fingertip.

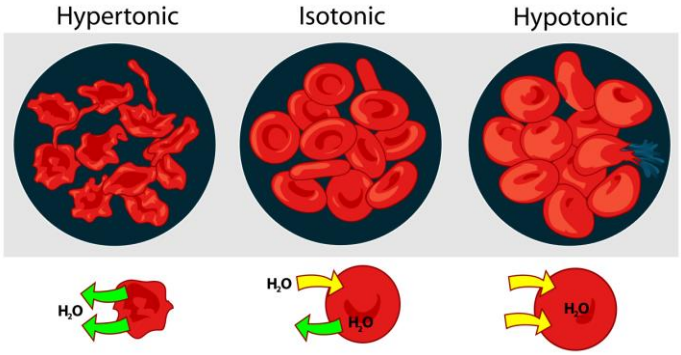
-After obtaining the blood sample, compress the gauze pad over the cut until bleeding ceases.

2.3.2. Slide preparations

-Use three clean microscope slides and three coverslips.

-Label the slides as 1, 2, and 3.

-Obtain a sample of fingertip blood, and use the finger to place a small amount in each of the slides.



-Immediately dilute the blood on the slide 1 with a drop of 5% saline, and apply a coverslip.

-Dilute the blood on slide 2 with one drop of physiologic saline and apply a coverslip.

-Dilute the blood on slide 3 with one drop of distilled water, and apply a coverslip.

-Examine each mixture under the normal light microscope. Observe the size, shape, and appearance of the red blood cells.

2.4. Results

-Have you observed RBS burst? In which part of the experiment?

-Have you observed RBC shrinkage? In which part of the experiment?

- Why you used 5% normal salin in the part 3 of your experiment?

2.5. Review questions

- What happened when you mixed a blood with hypotonic solution? Why?

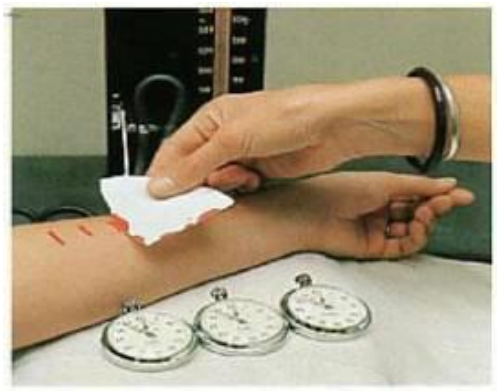
- What happened when you mixed a blood with hypertonic solution? Why?

- What happened when you mixed a blood with isotonic solution? Why?

- Why we performed this experiment on RBC and not WBC or other cells?

Experiment 3: Bleeding time and clotting time

3.1. Bleeding Time



3.1.1. Aims

1.Screening test for inherited and acquired platelet defects.

2. Diagnosis, treatment, and study of hemorrhagic diseases.

3.1.2. Background

The bleeding time test is a rather old method and indicated when other more reliable and less invasive tests for determining coagulation are not available. However, it is still to this date the most reliable way of assessing clinical bleeding in patients with uremia.

3.1.3. Principles

The bleeding time test is a useful tool to test for platelet plug formation and capillary integrity. Occasionally, the bleeding time test will be ordered on a patient scheduled for surgery. The bleeding time is dependent upon the efficiency of tissue fluid in accelerating the coagulation process, on capillary function and the number of blood platelets present and their ability to form a platelet plug.

3.1.4. Materials and Methods

1. Dukes method (Simpler and more easy).

2. Ivy's method (blood pressure cuff is used).

Equipments and supplies

1.Stopwatch.

2.Filter paper. 3. Automated incision making device or blood lancet. 4. Alcohol pad, tissue and cotton.

3.1.4.1. Procedure of Duke Method

1. Sanitize the lateral aspect of the forearm or ear lobe.

2. An incision of about 5 mm long X 1 mm deep should be made.

3. The blood should be blotted with filter paper every 30 seconds.

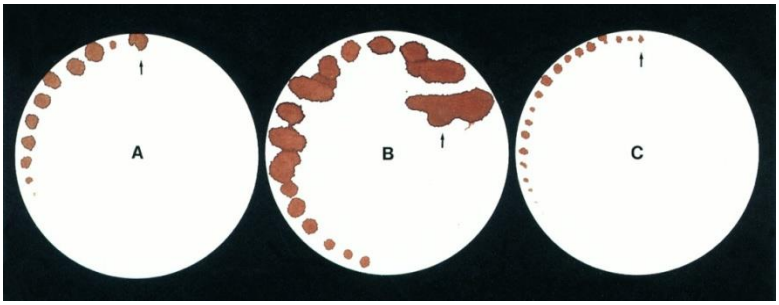
4. The time to cessation of bleeding is measured.

Note: If the bleeding time exceeds 15 minutes, stop the procedure.

Normal value: 2- 8 minutes.

3.1.5. Causes of prolonged bleeding

1. Vascular lesions (Vit C deficiency, bacterial and viral infections and hereditary conditions).



2. Platelet defects (thrombocytopenia).

3. Severe liver and kidney diseases.

4. Medication (coumarin, aspirin and heparin).

5. Clotting factor deficiency (hemophilia).

3.1.6. Source of error

1. Aspirin and aspirin containing compounds prolonged the bleeding. Thus, these drugs should be stoped 7-10 days prior to the test.

2. Improperly performed puncture (too shallow incision).

3.1.7. Results

-Why we used forearm or ear lobe?

-Why an incision of about 5 mm long X 1 mm deep should be made?

-Why Dukes method is more preferable?

3.1.8. Review questions

-What are precautions to perform a test on a patient diagnosed with liver or kidney diseases?

-If the patient has taken cumarin or aspirin? What is your recommendation to him/her?

-Is it possible to perform a test on hemophilia patients?

-What you do if the bleeding takes more than 15 minutes?

3.2. Clotting Time

3.2.1. Aims

Measuring the activity of common pathways of coagulation.

3.2.2. Background

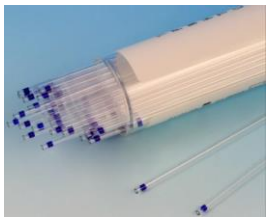
In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin then converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clotting factor, this process is also being assisted by Ca^{++} and by factors released by platelets and damaged tissues .

3.2.3. Principles

The time taken for blood to clot mainly reflects the time required for the generation of thrombin in this manner. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged.

3.2.4. Materials and Methods

1. Lee- white method.
2. Capillary tube method.

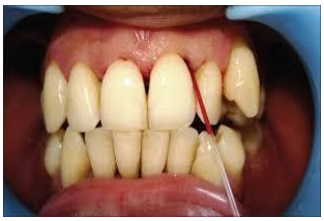


3.2.4.1. Capillary tube method.

Equipments and supplies

1.Stopwatch.

2. Capillary tube without anticoagulant. 3. Alcohol pad.



3.2.5. Procedure of capillary tube method

1. A skin puncture (thumb or gum) should be made and wipe away the first drop.

2. Fill a special capillary tube with blood.

3. Holding the tube between the thumb and index finger of both hands.

4. Gently break the tube every second until a strand of fibrin thread appears. The thread should be seen extending across the gap between the ends of the tube.

5. The interval between the appearance of the blood and the appearance of the fibrin is the coagulation time.

Normal value: The expected range for clotting time is 4-10 mins.

3.2.6. Causes of delay clotting

1. Deficiency in coagulation factors.

2. Vitamin K deficiency.

3. Thrombocytopenia. 4. Medications.

3.2.7. Results

-Why we used thumb or gum for blood sample collection? Is there any better areas to be selected?

- Why we chose capillary tube method? Is it better or cheaper or more accurate?

-Were you successful in both experiments?

3.2.8. Review questions

-Why calcium ions play a major role in coagulation pathway?

-What are the medications that affecting clotting time? Can patient have these medications before the test?

-What is the role of vitamin K level in this test? Why?

-If the voulanteer has been diagnosed with prothrombin difficiency. What will be suspected to happen?

Experiment 4: Measurment of blood pressure

4.1. Aims

-Blood pressure measurement is important because the higher your blood pressure is, the higher your risk of health problems in the future.

-If your blood pressure is high, it is putting extra strain on your arteries and on your heart. Over time, this strain can cause the arteries to become to become thicker and less flexible, or to become weaker.

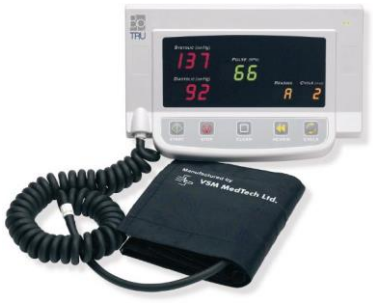
4.2. Background

-Blood pressure (BP) means measuring the pressure of the circulating blood that exerts on the walls of the arteries during the various stages of heart activity.

-A person's blood pressure is usually expressed in terms of the systolic pressure over diastolic pressure in millimeters of mercury (mm Hg).

4.2.1. Blood pressure measurement devices

1. Aneroid.
2. Mercury (Sphygomanometer).
3. Electronic.



4.2.2. Features

1- Ease of use: Electronic > Aneroid > Mercury

2- Cost: Electronic > Mercury > Aneroid

3- Accuracy: Mercury > Aneroid > Electronic

4- Memory: Electronic only

4.2.3. Types of blood pressure

1. Systolic

It measures the pressure in an artery when the heart is contracting (LUP-DUP).

2. Diastolic

It measures the pressure in an artery when the heart relaxes between contractions (DUP-LUP).

4.2.4. Importance of the blood pressure in our life

-Blood pressure is important because the higher your blood pressure is, the higher your risk of health problems in the future.

-If your blood pressure is high, it is putting extra strain on your arteries and on your heart.

-Over time, this strain can cause the arteries to become thicker and less flexible, or to become weaker.

-If your arteries become thicker and less flexible, they will become narrower, making them more likely to become clogged up.

-If an artery becomes completely clogged up, this can lead to a heart attack, a stroke, kidney disease or dementia.

-More rarely, if an artery has become weakened, the extra strain may eventually lead to the artery bursting.

4.3. Materials and Methods

4.3.1. Patient preparation before measurement

-No caffeine for 30 – 60 minutes.

-No smoking for 30 minutes.

-No exercise for 30 minutes.

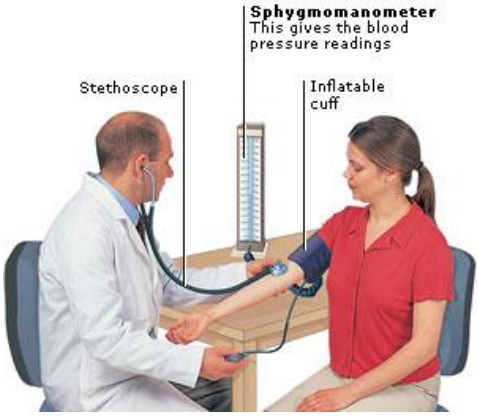
-Bladder/Bowel comfortable (empty).

-Quiet/temperate, relaxed environment, no talking.

-Bare arm with no constrictive clothing.

-The patient should stay silent prior and during the procedure.

-No acute anxiety, stress or pain.



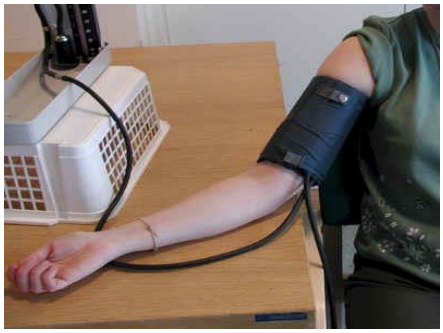
4.3.2. Position of the Patient

-Calmly seated for 5 minutes.

-Back well supported.

-Arm relaxed and supported at heart level.

-Legs uncrossed, feet flat on the floor and not dangling.



4.3.3. Arm preparation for measurement

-The measurements should be made with the right arm whenever possible.

-The patient's arm should be resting on the desk and raised.

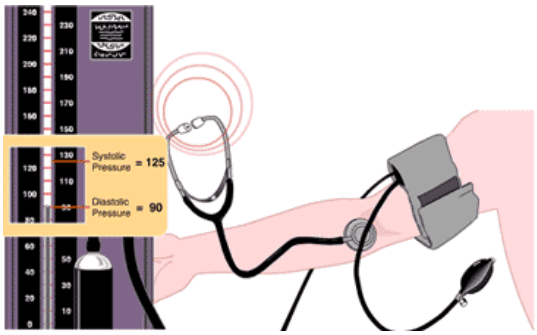
-Palm is facing up.

-The arm should remain somewhat bent and completely relaxed.

4.3.4. Procedure

1. Wrap the proper sized cuff around the patient's upper arm (be sure the cuff level is level with the heart) and loosen the thumbscrew.

2. With your left hand place the stethoscope head (diaphragm) directly over the brachial artery**.**



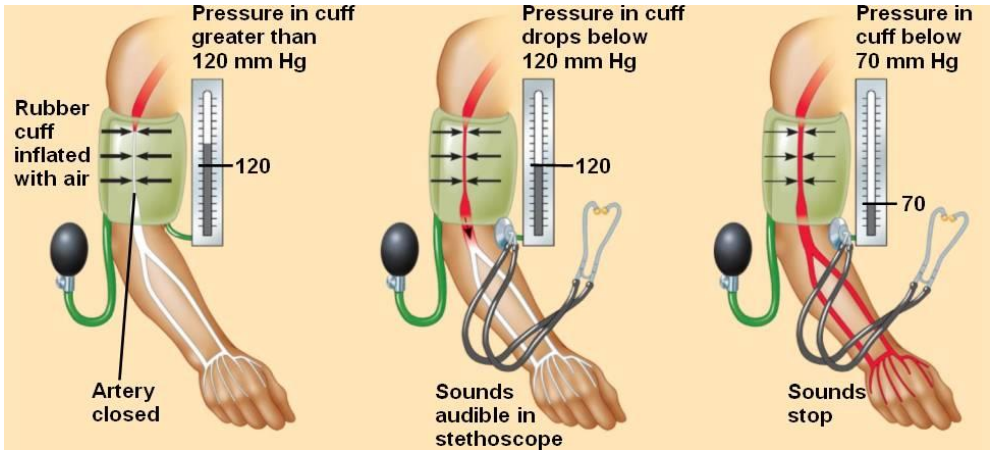
3. Use your right hand to pump the squeeze bulb several times to inflate the cuff and occlude the pulse (no longer feel the pulse).

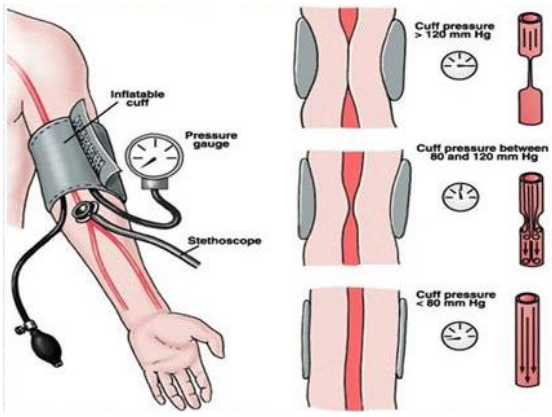
4. Measure systolic BP (once the sound disappeared).

5. Deflate the cuff gradually from the air by loosing the thumb screw till the appearance of the pulse again.

6. Measure diastolic BP (once the sound appeared).

-





Repeating the measurement

1. If you wish to repeat the BP measurement you should allow the cuff to completely deflate.

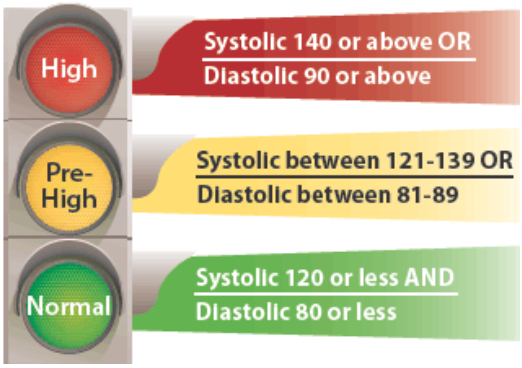
2. Permit any venous congestion in the arm to resolve.

3. Then repeat the measurement 3 minutes or a bit later.

Normal range

Systolic: 90-120mm Hg

Diastolic:60-80 mmHg



Blood pressure disorders

-BP that is pathologically low is called hypotension, and pressure that is pathologically high is hypertension.

-Both have many causes and can range from mild to severe, with both acute and chronic forms.

Hypotension (blood pressure below normal)

It may be indicated by a systolic pressure, lower than 90. It is characterized by dizziness and fainting.

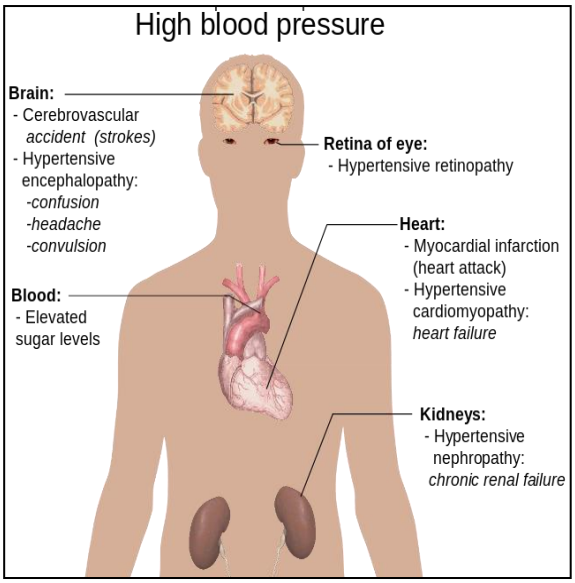
Causes of low blood pressure

1. Hemorrhage.

2. Toxins.

4. Hormonal abnormalities.

5. Eating disorders, particularly anorexia nervosa.



Hypertension (blood pressure higher than normal)

Blood pressure greater than 139/89.

Classification of Blood Pressure for Adults		
Category	**Systolic (mm Hg)**	**Diastolic (mm Hg)**
Hypotension	**< 90**	**< 60**
Desired	**90–119**	**60–79**
Pre-hypertension	**120–139**	**80–89**
Stage 1 hypertension	**140–159**	**90–99**
Stage 2 hypertension	**160–179**	**100–109**
Hypertensive emergency	**≥ 180**	**≥ 110**

Causes for development of hypertension

1. Obesity.

2. Poor dietary habits.

3. High sodium intake.

4. Sedentary lifestyle.

5. High alcohol consumption.

4.3.5. The common error during measuring BP

1. Incorrectly preparing the patient (over clothing, talking, smoking, eating, drinking, not resting,…….).

2. Emotional state (anxiety, stress, …….).

3. Technical errors (unsuitable sized cuff, wrong cuff or stethoscope placement, improper cuff deflation rate).

4. Observer bias, and faulty equipment.

5. Temperature (too low or too high).

4.3.6. Results

-Were you heard both systolic and diastolic sounds?

-Were you done the test in the lab successfully?

-How did you know the value you collected is correct?

4.3.7. Review questions

-Is it correct obesity people always have high blood pressure? Explain the effect of weight on BP.

-What happen if the test done in a very hot environment? What is your recommendation?

-Does stress and tension play a major role in measuring BP? How? and Why?

- What happen if the patient have caffeine before the test? What is your recomendtaon to him/her?

-Why the level of the tested arm should be at the level of the heart?

-What is the role of sodium on the BP?

Experiment 5: Cardiovascular Effects of Exercise

5.1. Aims

1. Strengthening muscles and cardiovascular system.

2. Honing athletic skills.

3. Weight loss or maintenance.

4. Enjoyment.

5. Help prevent depression.

6. Improve mental health generally.

8. It can augment an individual's sex appeal.

5.2. Background

Physical exerciseis any body movement that produced by muscle action to increase energy expenditure.It enhances or maintains physical fitness and overall health and wellness.

5.2.1. Types of physical exercise

1. Aerobic exercise

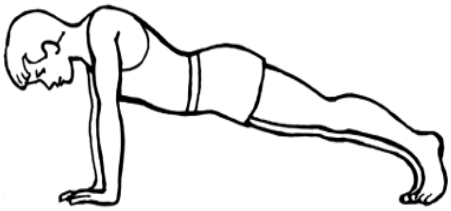
-It is any physical activity that uses large muscle groups and causes your body to use more oxygen than it would while resting to increase cardiovascular endurance.

-Examples of aerobic exercise include: cycling, swimming, walking, skipping rope, rowing, hiking, and playing tennis.

2. Anaerobic exercise (Strength or Resistance Training)

-It can firm, strengthen, and tone your muscles, as well as improve bone strength, balance, and coordination.

-Examples of strength moves are pushups, lunges, and bicep curls using dumbbells.



3. Flexibility exercise

-It stretches and lengthen your muscles.

-Stretching helps to improve joint flexibility and keep muscles limber.

-The goal is to improve the range of motion which can reduce the chance of injury.

5.2.2. Benefits of frequent/regular physical exercise

1. It boosts the immune system.

2. It helps prevent the heart disease, cardiovascular disease, Type 2 diabetes, and obesity.

3. It increases the blood and oxygen flow to the brain, growth factors that help neurogenesis.

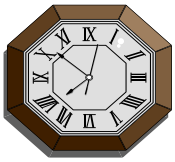
4. It increases chemicals in the brain that help cognition, such as dopamine, glutamate, norepinephrine, and serotonin.

5. It helps sleep disorders such as insomnia.

5.2.3. FITT Formula

-Frequency: Number of sessions each week.

-Intensity: Degree of effort spend by the individual duringexercise.



-Time: Duration of activity.

-Type: Mode of exercise being performed.

5.2.4. Body responses to exercise

1. The endocrine response to exercise, which include:

   a. Changes in glucose and fat metabolisms.

   b. Changes in fluid and electrolyte balance.

2. The cardiovascular response to exercise.

5.2.5. The endocrine response to exercise

5.2.5.1. Regulation of Glucose Metabolism During Exercise

-Glucagon secretion increases during exercise to promote liver glycogen breakdown (glycogenolysis).

-Epinephrine and Norepinephrine further increase glycogenolysis.

-Cortisol levels also increase during exercise for protein catabolism for later gluconeogenesis.

-Growth hormone mobilizes free fatty acids.

-Thyroxine promotes glucose catabolism.

-As intensity of exercise increases, so does the rate of catecholamine release for glycogenolysis.

-During endurance events the rate of glucose release very closely matches the muscles need.

-When glucose levels become depleted, glucagon and cortisol levels rise significantly to enhance gluconeogenesis.

-Glucose must not only be delivered to the cells, but it must also be taken up by the cells via insulin.

-Exercise may enhance insulin's binding to receptors on the muscle fiber.

-Up-regulation (receptors) occurs with insulin after 4 weeks of exercise to increase its sensitivity.

5.2.5.2. Regulation of fat metabolism during exercise

-When low plasma glucose levels occur, the catecholamines are released to accelerate lipolysis.

-Triglycerides are reduced to free fatty acids by lipase which is activated by cortisol, epinephrine, norepinephrine, and growth hormone.

5.2.5.3. Hormonal effects on fluid and electrolyte balance

-Reduced plasma volume leads to release of aldosterone, which increases Na+ and H2O absorption by the kidneys and renal tubes.

-Antidiuretic Hormone (ADH) is released from the posterior pituitary when dehydration occurs, and more water is then reabsorbed by the kidneys.

5.2.5.4. Cardiovascular response to exercise

1. Rapid onset of increasing pulse (heart) rate and systolic BP.

2. Decreasing or not changing of the diastolic BP.

-Stroke Volume (**SV**)

It is the volume of blood pumped from the left ventricle of the heart per beat.

-Factors increasing stroke volume

1) Increased Venous Blood

Trained individuals have enhanced blood return to the heart, which increases venous blood, therefore the heart can pump more blood.

2) Ventricular Stretch

This stretch leads to a more powerful ventricular contraction.

3) Ventricular Contractility

The heart is a muscle, and has the capacity to contract during training with greater force production, thus ejecting more blood.

4) Aortic and Pulmonary Artery Blood Pressure

Healthy arteries have better vasodilatation, allowing blood to travel much more efficiently through the systemic circuit.

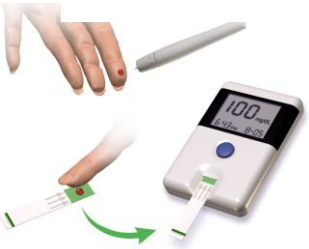
-Thus, systolic blood pressure increases with exercise intensity.

-During exercise, diastolic pressure should stay pretty stable in healthy individuals, sometimes it actually drops due to the vasodilatation of arteries.

5.2.6. Materials and Methods

1. Sphygomanometer to measure blood pressure.

2. Oximeter to measure pulse rate and ECG.



3. Thermometer to measure temperature.

4. Blood sugar test stips for measuring of glucose.

5.2.7. Procedure

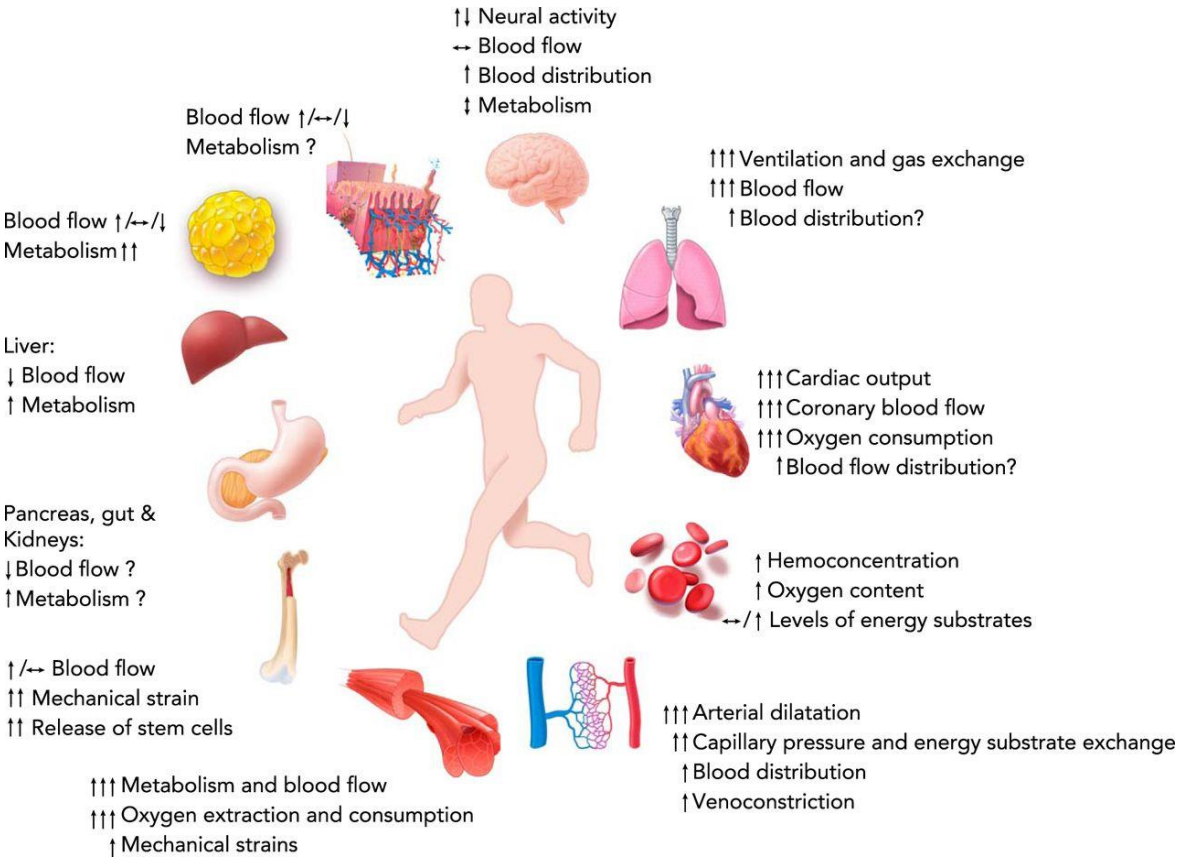
1. Measure the pulse rate, blood pressure, temperature and blood glucose before exercise.

2. Start the exercise for not less than 10-15 minutes.

-The exercise may be done on the treadmill or by running in the corridor.

3. Measure the pulse rate, blood pressure, temperature and blood glucose after exercise.

4. Compare the results before and after exercise to know what is the cardiovascular and endocrine responses to the exercise.



-Excessive exercise

-Too much exercise can be harmful without proper rest.

-The chance of stroke or other circulation problems increase.

-Muscle tissue may develop slowly.

-Extremely intense, long-term cardiovascular exercise, has been associated with scarring of the heart and heart rhythm abnormalities.

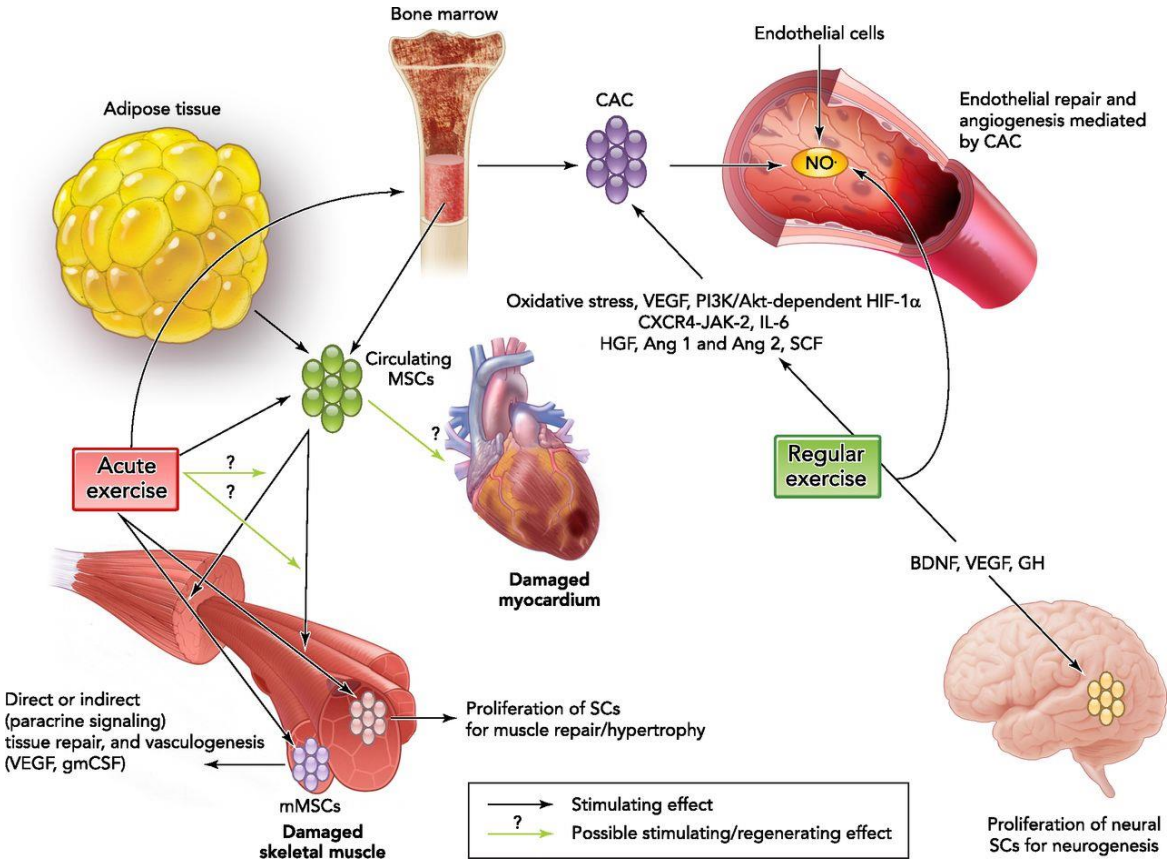
-Cardio-respiratory endurance

-Frequency: 3 to 5 times per week.

-Intensity: 60% to 90% $HR_{MAX.}$

-Time: 20 - 30 minutes.

-Type: Aerobic activities (Jogging, running, walking, dancing, biking, swimming).



5.2.8. Results

-Have you seen any differences in pulse rate and blood pressure values before and after exercise? Why?

-Have you observed any differences between indoor and outdoor exercises?

-Are you agree with your collected data?

5.2.9. Review questions

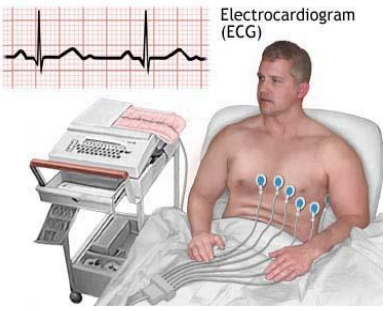
-Do you think that exercise has antidepression effects on those diagnosed with clinical depression?

-Are there any changes in blood parameters especially hemogram after exercise?

-How can you perform a regular exercise? Explain?

-Does exercise has any effect in diabetic patients? How?

Experiment 6: Electrocardiogram (ECG) and heart sounds



6.1. Aims

1. Routine screening of cardiac pathologies.

2. Immediate assessment of patients suffering from chest pain.

3. To diagnose the presence of heart diseases (myocardial infarction and cardiac dysrrhythmias).

4. To diagnose the presence of cardiac enlargement and size of the cardiac chambers.

5. To evaluate the effect of therapeutic interventions on the heart (drugs, fluid, and mechanical support).

6.2. Background

- ECG is a quick, simple, painless procedure in which the heart's electrical impulses are amplified and recorded on a piece of paper.

- ECG provides information about the part of the heart that triggers each heartbeat, the nerve conduction pathways of the heart, and the rate and rhythm of the heart.

-The ECG is a major diagnostic technique for the assessment of the health of the heart.

-This measurement is taken at the surface of the skin which reflects the electrical phenomena in the heart when the SA node triggers the electrical sequence that controls heart action.

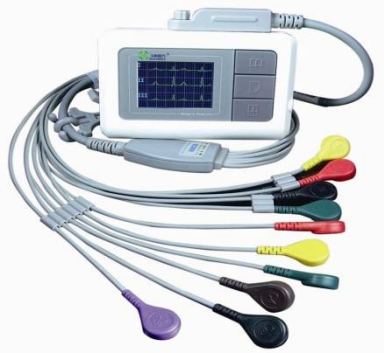
-Electrocardiograph

It is the instrument that measures the electrical activity of the heart on the body surface and used in the detection and diagnosis of heart abnormalities.

- Electrocardiogram (ECG)

It is the record of that activity or it is a test that checks for problems with the electrical activity of the heart. It translates the heart's electrical activity into line tracings on paper.

6.3. Materials and Methods



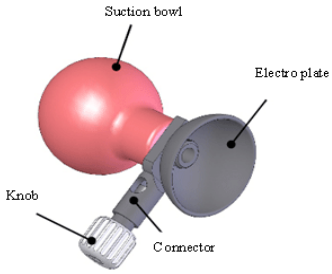
1. ECG machine, cables, leads (electrodes) with sensor, and graph paper.

2. Electrode paste or gel.

3. Alcohol wipes.

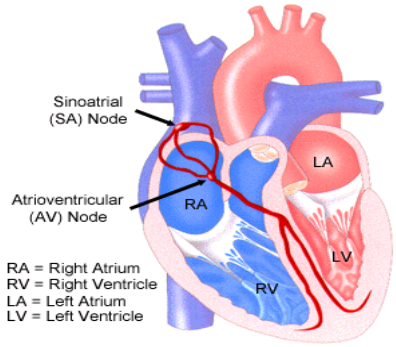
4.Pillow, sheet, towel or drape if necessary.

5.Disposable razor (especially for male).



6.3.1. The Heart's Electrical Sequence

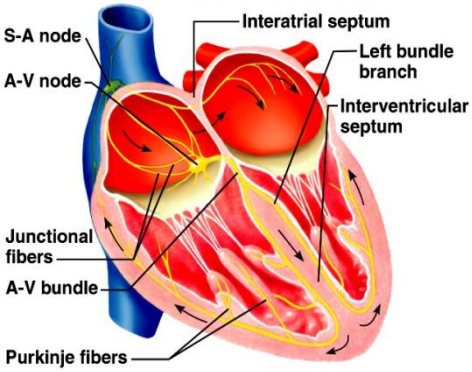
-A specialised electrical conducting system in the heart ensures an orderly contraction, so that the heart can act as an efficient pump.



-Below the right atrium is the sinoatrial (SA) node, an area of specialized muscle fibers that propagates the heart's contraction stimulus.

-It has the ability (in the absence of external stimuli) to initiate electrical impulses at a rate of approximately 100/minute.

-The synchronized electrical sequence of the heart is initiated by the SA node, the heart's natural pacemaker.



-The firing of the SA node sends out an electrical impulse via its neurons to the right atrium, left atrium, and AV node simultaneously.

-Since the right atrium is closer to the SA node, it depolarizes first, resulting in pumping

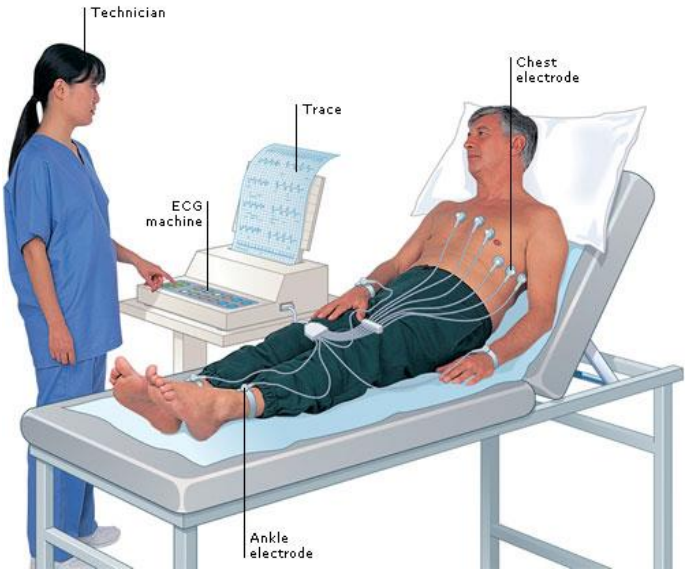
action by the right atrium before the left atrium.

-At the AV node, the impulse is delayed to allow for the ventricles to fill up with blood.

-After the delay, the AV node sends the impulse to the Bundle of His and the Purkinje fibers.

-This triggers the contraction of the ventricles to send blood either to the lungs or out to the body.

6.4. Procedure

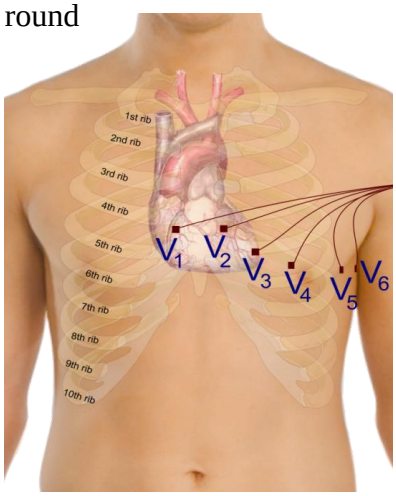


1.Lay down the patient in a semi-prone position comfortably with shirts, blouses, bras and socks removed.

2.If the patient is male and the chest is particularly hairy, it will need to be shaved.

3.Put the electrode paste or gel on the skin.

4.Connect the leads (electrodes). Place electrodes (small round sensors that stick to the skin) on the person's arms, legs, and chest. These electrodes measure the magnitude and direction of electrical currents in the heart during each heartbeat.



5. Run the device and record the result.

6.4.1. Positioning of Leads

-V1 4th intercostals space on the right sternal border.

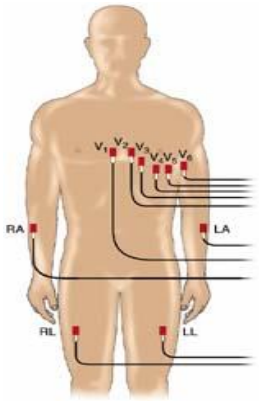
-V2 4th intercostals space on the left sternal border.

-V3 between V2 and V4.

-V4 5th intercostal space on the mid clavicular line.

-V5 between V4 and V6 on the same horizontal plane.

-V6 mid axilliary on the same plane as V4 and V5.



-Right arm lead (RA).

-Left arm lead (LA).

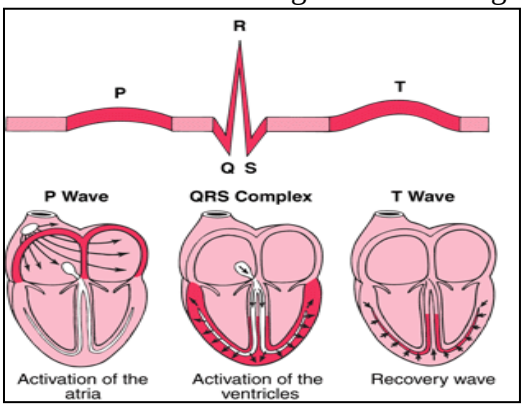
-Right leg lead (RL).

-Left leg lead (LL).

-Notes

-The electrodes are connected by wires to a machine, which produces a record (tracing) for each electrode.

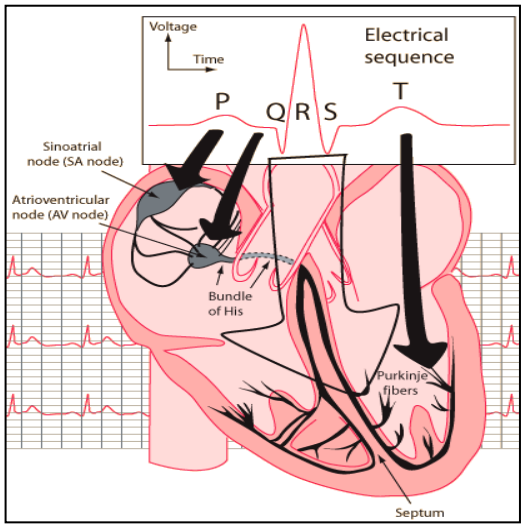
-Each tracing shows the electrical activity of the heart from different angles. The tracings constitute the ECG that takes about 3 minutes, is painless, and has no risks.



-If the lines of the tracing appear slightly blurred, the filter should be applied.

-Press the button according to the make of the ECG machine to run off a hard copy of the tracing.

-Document in the patient notes and on the computer that the ECG has been done.



-The ECG machine should be left in charge and disconnected from the mains prior to use as per manufacturers guidelines.

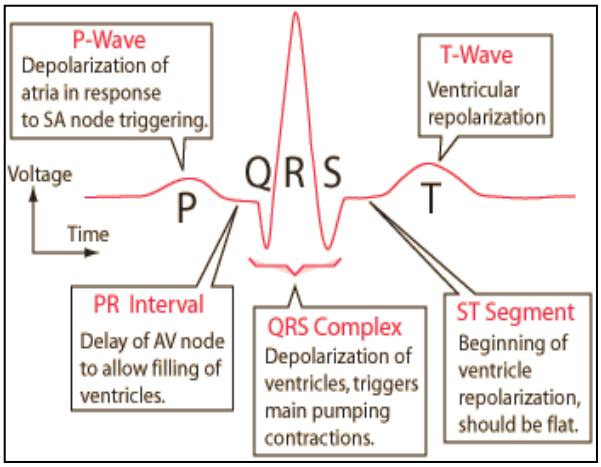
-The ECG machine should be serviced yearly.

6.4.2. Reading of the Result

-ECG represents the electrical current moving

through the heart during a heartbeat.

-The current's movement is divided into parts, and each part is given an alphabetic designation in the ECG.



-Each heartbeat begins with an impulse from the heart's pacemaker (sinus or SA node).

-This impulse activates the upper chambers of the heart (atria).

-The P wave represents activation of the atria.

-Next, the electrical current flows down to the lower chambers of the heart (ventricles) and make the QRS complex.

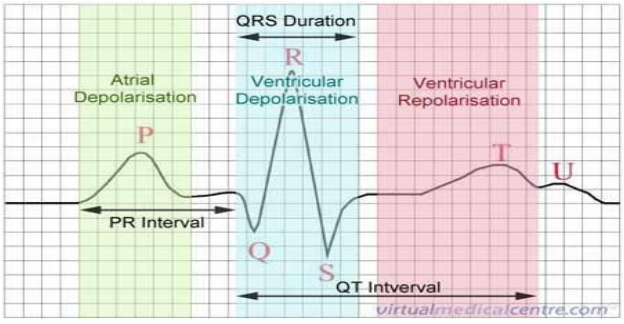
-The electrical current then spreads back over the ventricles in the opposite direction. This activity is called the recovery wave, which is represented by the T wave.

6.4.3. Components of the Electrical Sequence

-The spikes and dips in the line tracings are called waves.

-P Wave: Firing of the SA node and depolarization of the atria.

-PR Interval: Delay of the electrical impulse at the AV node and the depolarization of the atrium.



-QRS Complex: Ventricular depolarization.

-Q-wave = first negative deflection.

-R-wave = first positive deflection.

-S-wave = second negative deflection.

-ST Segment: The beginning of ventricular repolarization. It should be isoelectric and flat at baseline.

-T Wave: Ventricular repolarization.

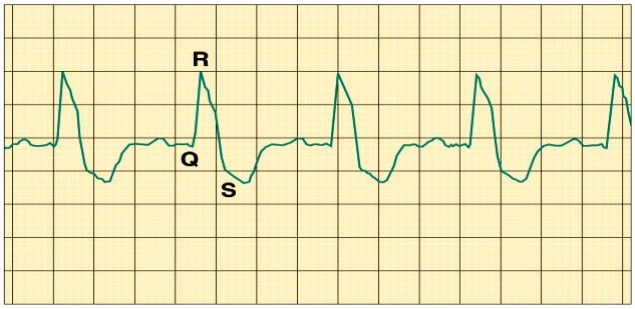
6.4.4. Electrical Properties of the Heart

-Pacemaker

It means the electrical generation of the heart that started from SA node.

-Action potentials

It is a rapid depolarization followed by rapid, partial early repolarization. Action potentials through myocardium during cardiac cycle produces electric currents than can be measured.



6.4.5. Sources of Error

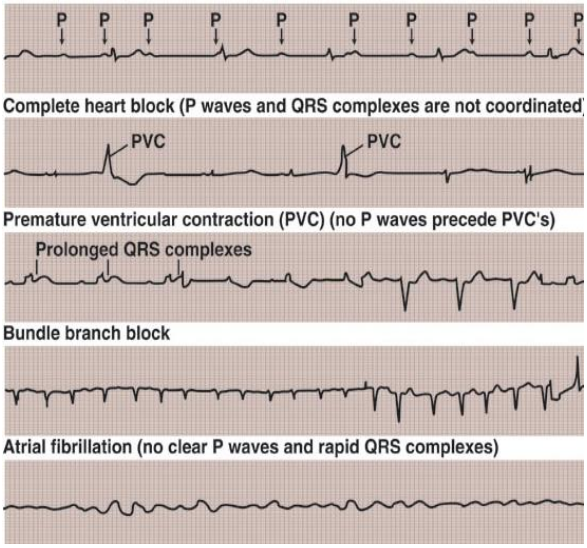
1. Improper skin preparation.

2. Wrong placement of the electrodes.

3. Old, broken or inaccurate ECG machine.

4. Unskillful physician.

5. Wrong interpretation of the result.



6.4.6. Cardiac Abnormalities

-Tachycardia: Heart rate in excess of 100 bpm.

-Bradycardia: Heart rate less than 60 bpm.

-Arrhythmias are problems with the rate or rhythm of the heartbeat.

-Premature atrial contractions: Occasional shortened intervals between one contraction and succeeding, frequently occurs in healthy people.

Artificial Pacemaker

It is a small device that's placed in the chest or abdomen to help control abnormal heart rhythms. This device uses electrical pulses to prompt the heart to beat at a normal rate.

6.4.7. Results

-Could you conduct the procedure successfully? If not, why?

-In case of unsuccessful, try your best to repeat the experiment again.

6.4.8. Review questions

-In case of tachycardia, how will be QRS reading?

- In case of arrythmias, how will be QRS reading?

-Is there any differences in ECG in normal people and in those with artificial pacemaker? Why?

-What are the foods and drinks that should never being taken before doing ECG?

-Do you think there is variation in reading ECG result on different times of the day? If yes, what time is better?

-Is there any hormonal relationship to ECG result?

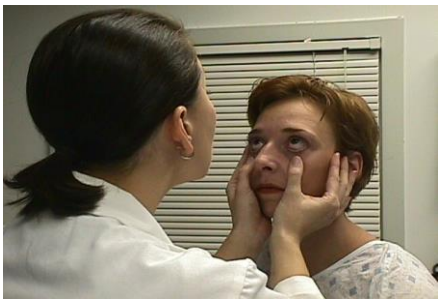
Experiment 7: Clinical examination of vision

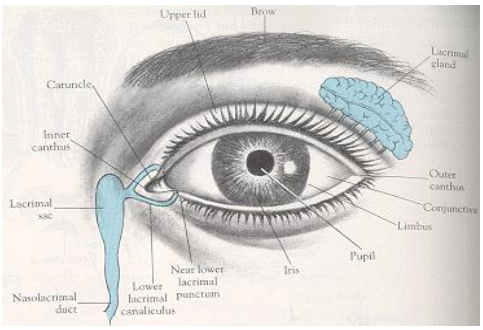
7.1. Aims

1. To inspect external ocular (eye) structures such as eyelids, conjunctiva, sclera, iris, lens and cornea.

2. To check the internal eye responses such as visual acuity, visual field, convergence insufficiency, pupillary response and colorblindness.

3. To determine vascular disorders of the eye, as in the case of glaucoma.





7.2. Background

An eye examination is a series of tests performed by an ophthalmologist assessing vision and ability to focus on discern objects, as well as other tests and examinations pertaining to the eyes.

7.2.1. Anatomy of the Eye

1. Pupil

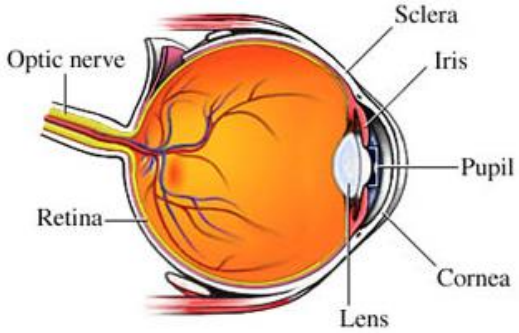
2. Cornea

3. Iris

4. Sclera

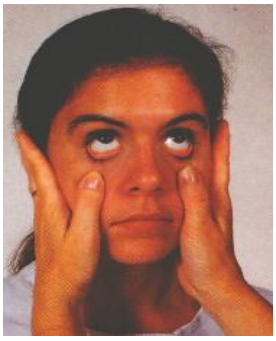
5. Retina

6.Lens 7. Optic nerve



7.2.2. External Inspection

-It is done by sliding the lower eyelids down, observe for redness which may be pathogenic.



-Healthy eyeball looks moist and glossy.

-Conjunctiva

-Conjunctivitis is the inflammation of the conjunctiva which may be due to bacterial, viral, allergic, or chemical irritation.

- Conjunctivitis is characterized by redness throughout the conjunctiva, but usually clear around the iris.

Conjunctivitis

-Sclera

-It should be white, although may have gray-blue hue.

-Yellowing of sclera indicates jaundice.

-Iris

-Iritis is characterized by red halo around the iris and cornea.

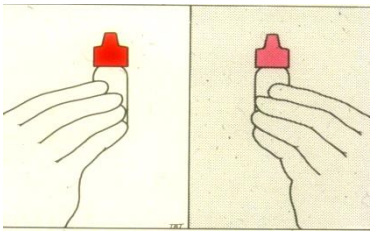
-Symptoms are photophobia, blurred vision, throbbing pain.

-Lens

-Cataract is an opacity of the normally clear lens.

-It may develops as a result of aging, metabolic disorders, trauma or hereditary.

7.2.3. Internal Inspection



-Visual Acuity

-It is an acuteness or clearness of vision.

-A common cause of low visual acuity is a refractive error, or errors in how the light is refracted in the eyeball.

-Too high or too low refractive error is the cause of nearsightedness (myopia) or farsightedness (hyperopia).

-These anomalies can mostly be corrected by optical means (such as eyeglasses, contact lenses, laser surgery, etc.).

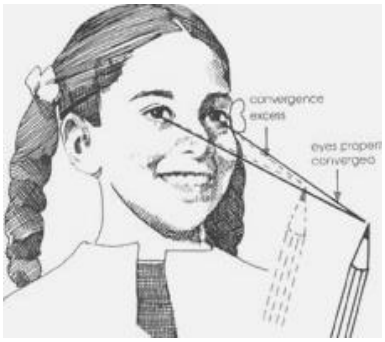
-Normal refractive status is referred to as emmetropia.

-Visual Field

-It is the portion of the subject's surroundings that can be seen at one time.

-The normal extent of the field of vision is 50° superiorly, 60° nasally, 70° inferiorly and 90° temporally.

-Convergence Insufficiency

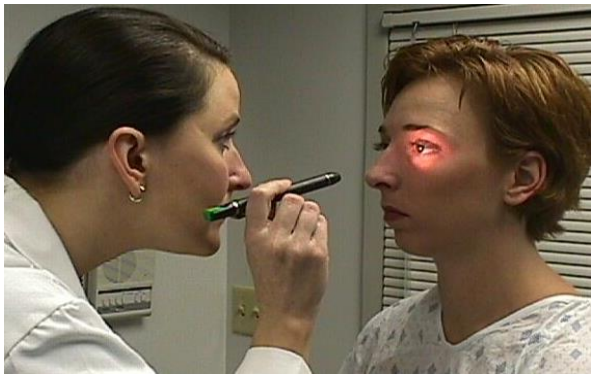


-It means that the eyes don't work together while trying to focus on a nearby object.

-When read or look at a close object, eyes need to turn inward together (converge) to focus.

-This gives binocular vision, enabling to see a single image.

-Pupillary Response



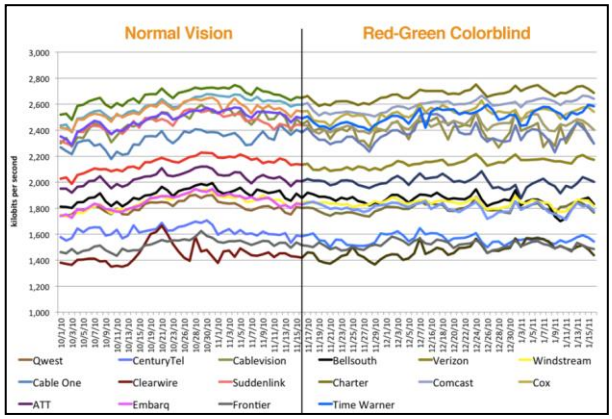
It means test the papillary response to light by observing the symmetrical reflection of pen light in both pupils.

-Direct response: pupil constricts in examined eye.

-Indirect (Consensual) response: pupil constricts in the opposite eye.

-Colorblindness

- It is the inability or decreased ability to see color, or perceive color differences, under normal lighting conditions.

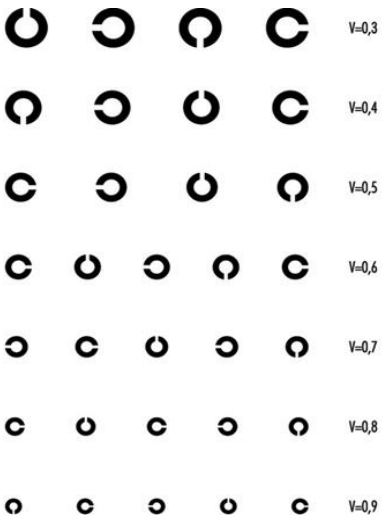


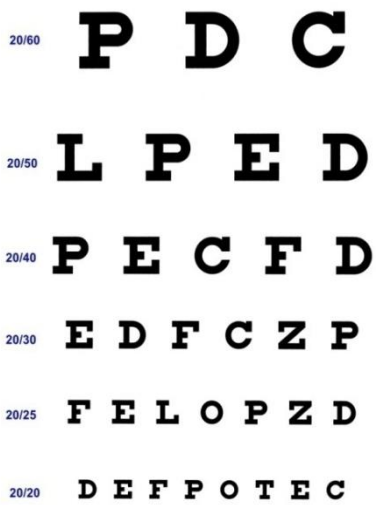
-The most usual cause is a fault in the development of retinal cones that perceive color in light and transmit that information to the optic nerve.

7.2.4. Snellen Chart

-It is an eye chart that can be used to measure visual acuity.

-The common Snellen chart is printed with eleven lines of block letters. The first line consists of one very large letter, which may be one of several letters, for example E, H, or C. Subsequent rows have increasing numbers of letters that decrease in size.





7.3. Procedure

1.Test your visual acuity with correction (contact lenses or glasses).

2.Stay far about 9 feet or 3 meters from the chart.

3.Test one eye at a time. Start with the right eye, covering the left one without pressing on it. Then, examine the left eye by doing the opposite.

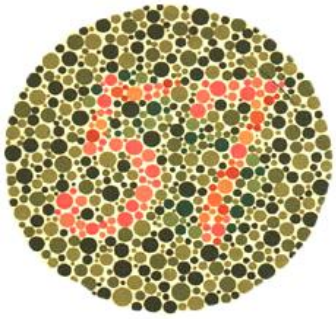
4.Read the letters from the largest to the smallest.

-To make the examination easier and faster, another person can help you by showing the letters you must read between the lines of letters.

5. If you can read the letters of the 8th line, your sight is optimal (visual acuity 20/20).

- If you have doubts about your sight, visit your ophthalmologist.

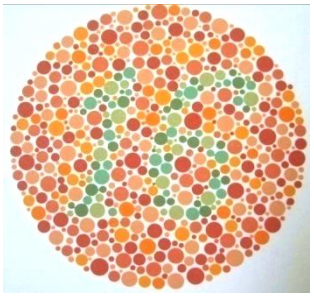
Ishihara Test



-It is a color perception test for red-green color deficiencies.

-The test consists of a number of colored plates, called Ishihara plates, each of which contains a circle of dots appearing randomized in color and size.

-Within the pattern are dots which form a number or shape clearly visible to those with normal color vision, and invisible, or difficult to see, to those with a red-green color vision defect.



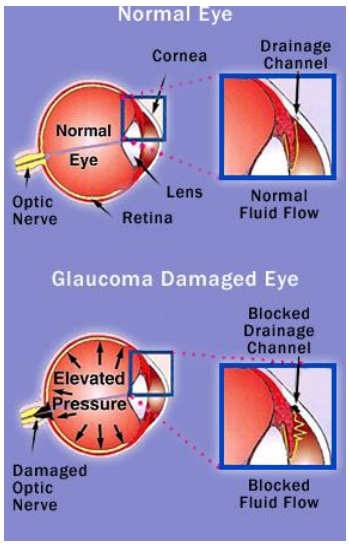
7.3.1. Procedure

1. Sit approximately 75 cm from the ishihara chart.

2. Preferably have mild natural light and no glare on your screen.

3. Attempt to identify the hidden number or line within 5 seconds, then click on the image.

4. Continue to the next Ishihara test, Complete them all to help gauge your color blindness severity.



7.3.2. Vascular Disorder of the Eye

-Glaucoma is caused by excessive pressure in eye due to blockage of outflow from anterior chamber, which puts pressure on optic nerve.

-It is characterized by redness around the iris and dilated pupils.

-Symptoms are sudden clouding of vision, sudden eye pain, and halos around lights.

7.3.3. Results

- Is different colors of ishihara chart affects the reading of your result?

- Could you perform visual acuty test? How? What was your result?

-How could you perform pupillary response test? Explain

7.3.4. Review questions

-How long an individual should visit ophthalmologist to check for internal and external eye inspection?

-What are the problems whith the eye of individuals diagnosed with colorblindness?

-What are the abnormalities of the external parts of the eye? Which one is most common?

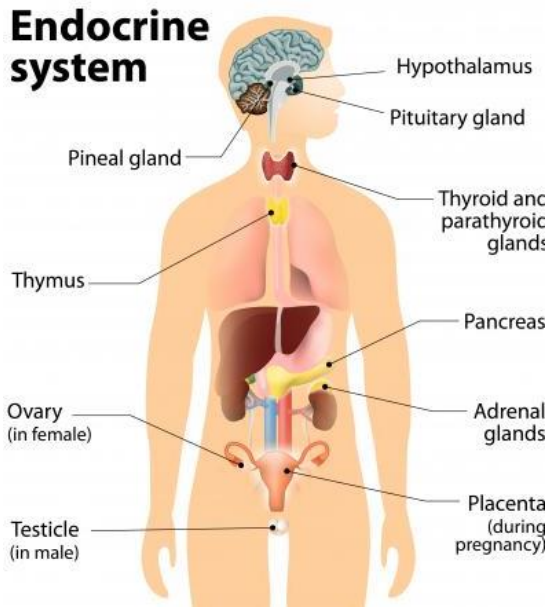
-What are the abnormalities of the internal parts of the eye? Which one is most common?

-Is there any relationship between heavy sleepless and eye abnormality? How?

Experiment 8: Pancreas function and insulin shock

8.1. Aims

To determine the effect of glucose on body metabolism and activity in living organism.

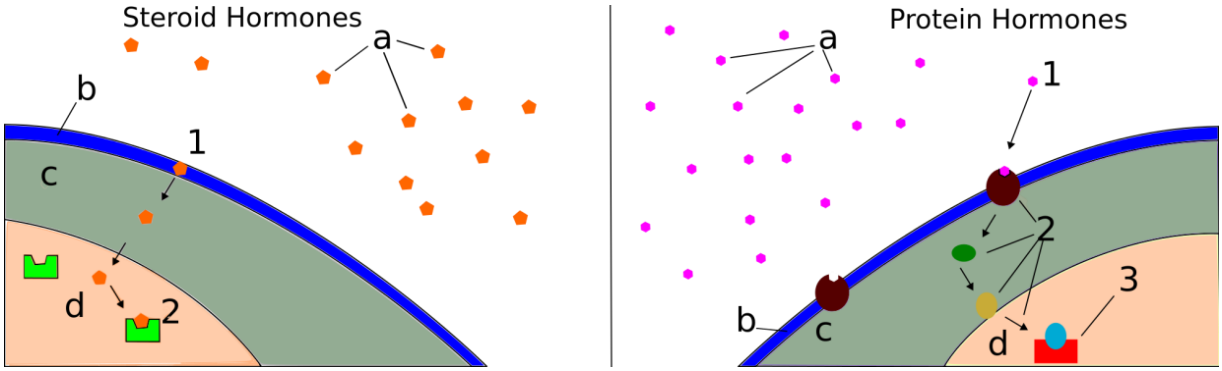


8.2. Background

-The endocrine system is the second major controlling system of the body after nervous system.

-The role of the endocrine system is to alter the activity of body cells primarily through chemical changes induced through the glands of the endocrine system.

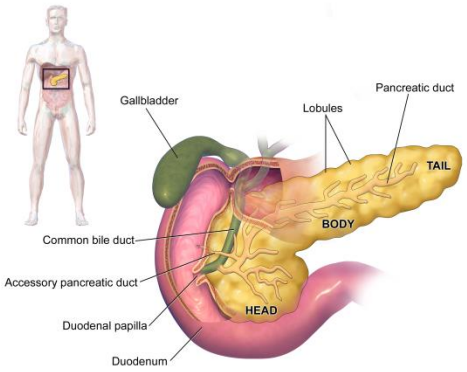
-These chemical "messengers" are referred to as hormones, and are typically released into the blood for transport throughout the body.



-A given hormone affects only the biochemical activity of selected organ or system.

-Those tissues that respond to the effect of the hormone are referred to as the target tissues or organs.

8.2.1. Pancreas and Insulin

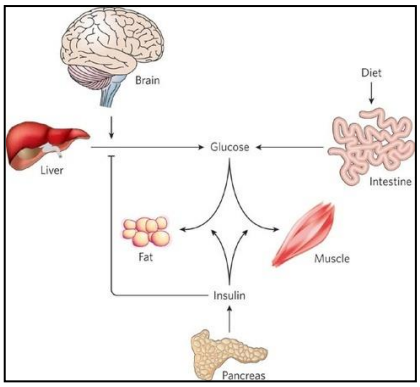


-It is a hormone produced by the pancreas to decrease blood glucose levels, and is found in virtually all vertebrates.

-When food is consumed and digested, glucose molecules enter the bloodstream and if enough food is eaten in a small period of time, elevation of blood glucose can occur.

-Without insulin, the levels of blood glucose can reach such elevated levels that there is great risk for cellular and tissue damage, especially within tissues of the nervous system.

-Likewise, too much insulin in the body will cause glucose levels to plummet below normal levels.

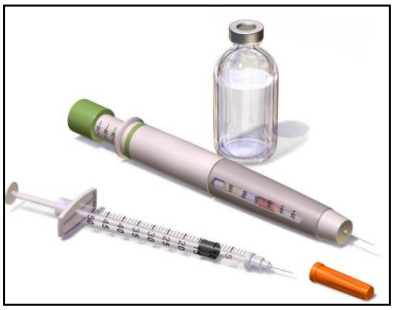


- If the drop in insulin levels is not too severe, the organism will experience mild symptoms of fatigue, anxiety, tremors, and hunger.

-However, if the drop in glucose through very high levels of insulin grows more extreme, the organism can experience insulin shock.

8.2.2. Insulin Shock

-It isan acute physiological condition resulting from excess insulin in the blood.



-It occurs when the blood glucose levels drop (hypoglycemia) to such low levels that the brain is no longer sufficiently provided with glucose and begins to malfunction.

-Symptoms of insulin shock include disorientation, convulsions, unconsciousness, and if left untreated will result in death.

-Fortunately, the insulin shock is easily reversed by providing a rapidly metabolized form of glucose to those affected.

8.2.3. Materialsand Methods

1. Goldfish
2. Insulin
3. Syringes with needles.
4. Ice
5. Beakers
6. Timer
7. Glucose solution

8.2.4. Procedure

8.2.4.1. Experiment-1

1. Fill a beaker to a halfwith water.

2. Place one goldfish in the beaker. Allow it to acclimate for 5 minutes.

3. Begin observing the behavioral patterns of the fish in the following orderfor a period of 2 minutes: mouth gaping, operculum movements and pectoral fin movements.

8.2.4.2. Experiment-2

1. Into the beaker of fish in the experiment above, add 1 ml of insulin .

2. Repeat step 3 (in experiment 1) and record the behaviors of the fish.

3. Pour into the beaker, 50 ml of glucose solution. After waiting 2 minutes, again record the behaviors of the fish.

8.2.4.3. Experiment-3

1. Place the beaker in a bowl containing a quantity of ice.

2. Repeat the steps 1–3 for insulin exposure.

3. Record the temperature of the water in which the fish is residing.

8.2.5. Results and Interpretation

There are three very distinct behavior patterns that can be observed in goldfish as follows:

1. Mouth gaping behavior

It is an indication of the fishes' metabolism. As the metabolism of the fish increases, the mouth gaping behavior increases in goldfish.

2. Operculum movement

It is a behavior that signifies oxygen consumption in the animal. As the number of operculum movements increases in a given unit of time, it is associated with decreased overall oxygen within the body.

3. Pectoral fin movement behavior

The movement of the fins are vital for balance, direction, and maintenance of position in the fish. The number and quality of the fin movement signifies the overall physical state of the fish.

8.3. Review questions

-What are the causes of hyperglycemia?

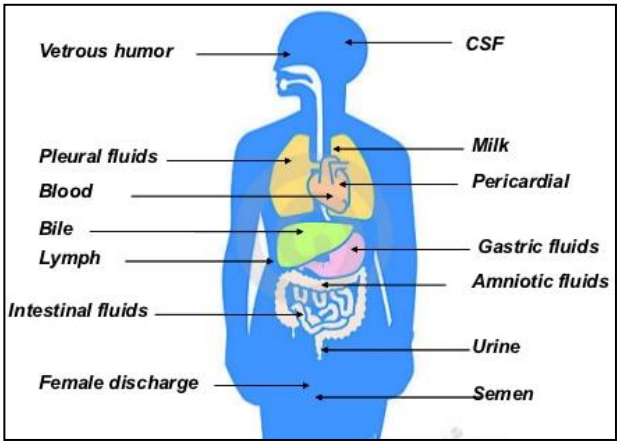
-What is the effect of liver on body glucose metabolism?

-Do you think which one is more dangerous: hypoglycemia or hyperglycemia?

-Why most of the aged peoples are suffering from hyperglycemia? Explain.

Experiment 9: Examination of body fluids

9.1. Aims

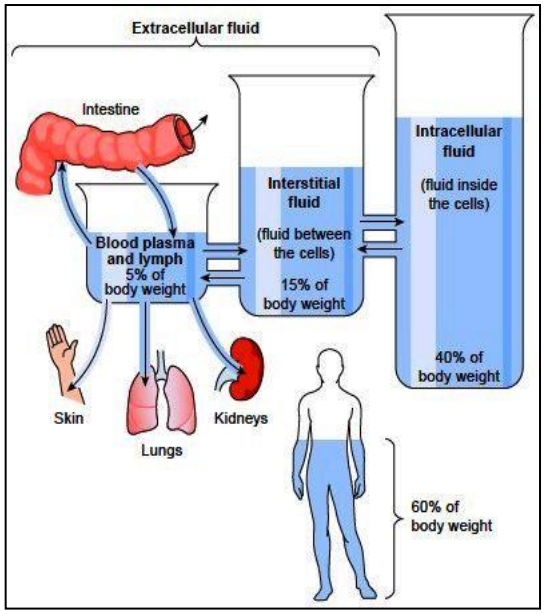


1. It is very useful for diagnosis of premalignant, malignant tumors and metastatic tumors.

2. It is also useful for diagnosis of inflammatory conditions (such as TB).

3. To check for general health condition.

9.2. Background



- Body fluids are liquids originating from inside the bodies of living organisms.

-The dominating content of body fluids is body water.

-Approximately 60-65% of body water is contained within the cells (intracellular fluid) with the other 35-40% of body water contained outside the cells (extracellular fluid).

-Body fluids are widely recognised as vehicles for the transmission of human disease.

9.2.1. Types of Body Fluids

They include fluids that are excreted or secreted from the body, such as:

1. Blood and lymph.

2. Urine, semen, tear, sputum, mucus and sweat.

3. Ceriebro spinal fluid (CSF), synovial fluid, and cerumen.

4. Saliva, milk, and bile.

5. Pleural, peritoneal, pericardial and amniotic fluids.

9.2.2. Factors Affecting Body Fluids

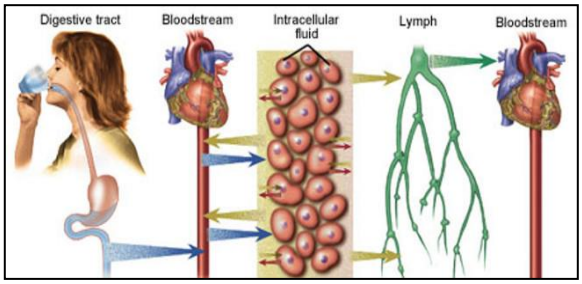
1. Water intake & output.

2. Age (infant: 73% whereas elderly: 45%).

3. Sex (adult male: 60% whereas adult female: 40-50%).

4. Climate.

5. Physical activity.



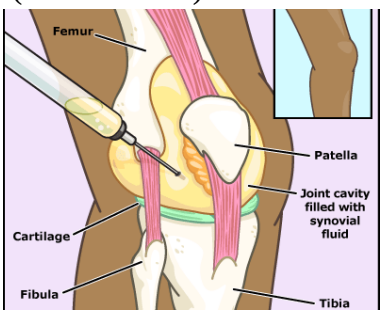
9.3. Sample Collection

-Amount: 1-5 ml is adequate.

-Storage: Specimen should be maintained at room temperature.

-Time relapse: Specimens should be processed immediately (less than 1 hr).

9.3.1. Methods of Sample collection

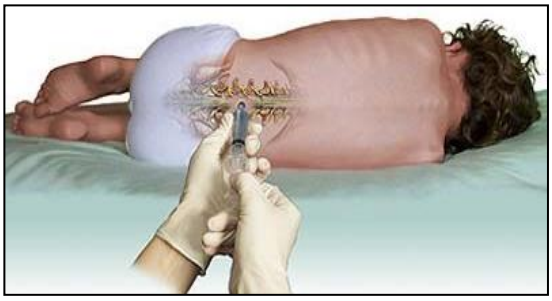


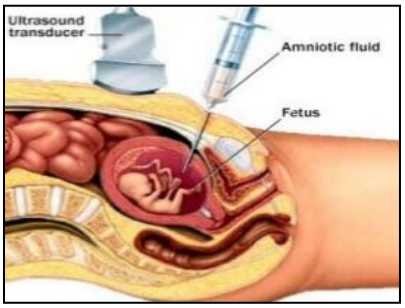
1. Direct Collection

It meansdirect aseptic collection of the fluid in a sterile, disposable, plastic container (urine, semen, milk, etc).

2. Indirect Collection

By inserting a sterile needle into the body cavity aseptically and aspirating with a syringe a portion of the fluid (CSF, synovial fluid, pericardial fluid,…….).





9.4. Tests for Body Fluids

A variety of tests may be performed, including:

1. Physical test

It means macroscopic (gross) examination of the color, odor, transperancy, as well as for pH and volume of the specimen.

2. Microscopic examinations

a. Direct smear for urine and milk.

b. Direct wet preparation by using 10% KOH for visualization of fungi element especially on vaginal discharge, saliva (tongue with Candidiasis) and skin specimens.

c. Staining technique such as Acid Fast stain for Mycobacterium spp. in sputum.

-In general if one organism is seen per oil immersion field, it means at least 10^5 organisms per milliliter of specimen are present.

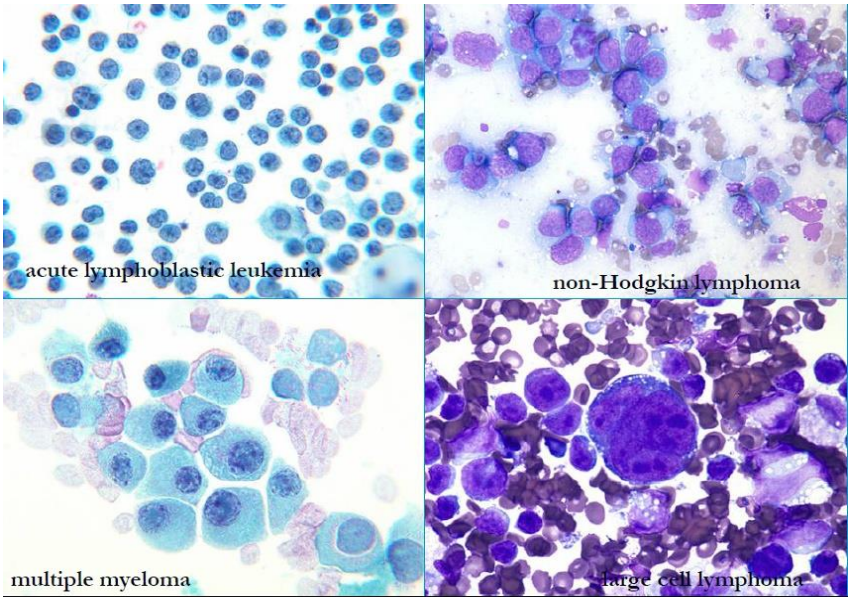
-3.Culturing or infectious disease tests

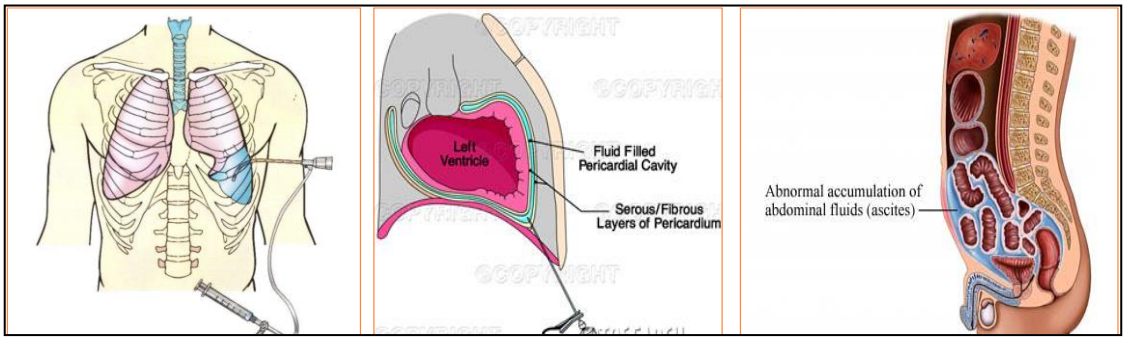
The collected specimen should be concentrated by centrifugation, and the resulting sediment should be inoculated to an enrichment broth, blood, chocolate and MacConkey agars. Then should be incubated for known period in a suitable environment.

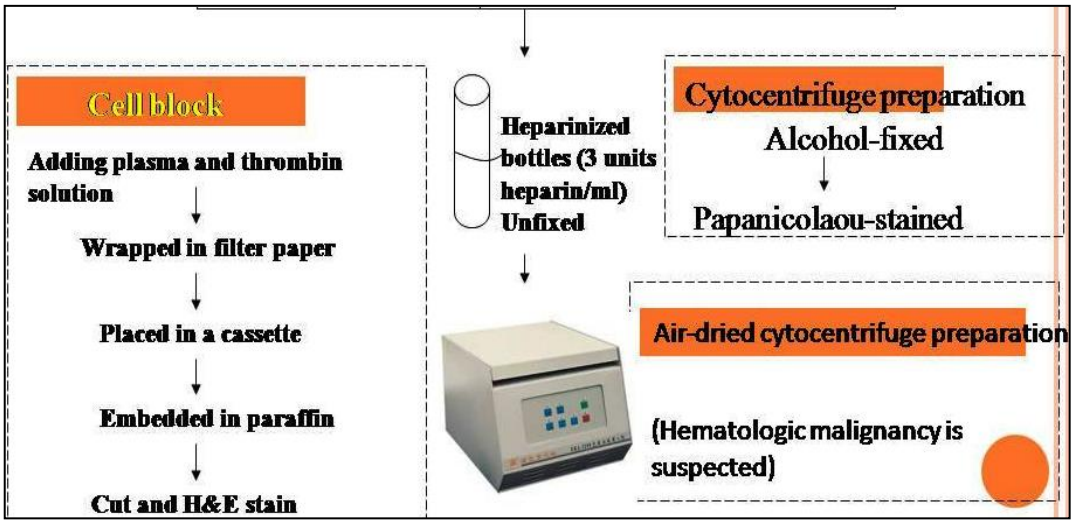
4. Total cell count and cytology

-It is performed especially for effusions (pleural, peritoneal, synovial, etc).

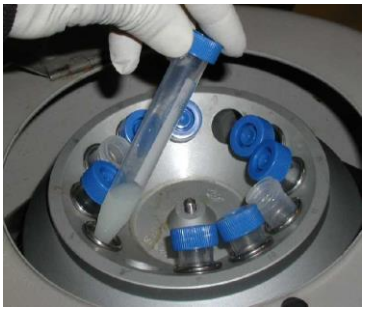
-0.1ml of heparin + 10ml of fluid should be mixed to prevent clotting.

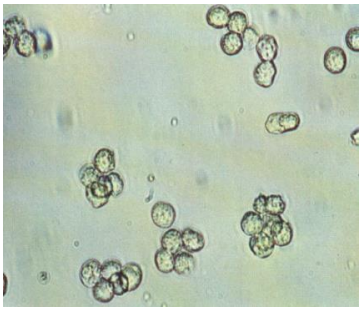






9.5. Microscopic Examination of the Urine





9.5.1.1. Procedure

1. Spun about 5 ml of the collected urine in a centrifuge at 2000rpm/5 minutes.

2. Throw the supernatant and spread the sediment on a slide, then coverslip.

3. Examine under low power of a normal light microscope.

9.5.1.2. Result Interpretation

a. Healthy

-No bacteria, yeast cells, squamous cells, or parasites are

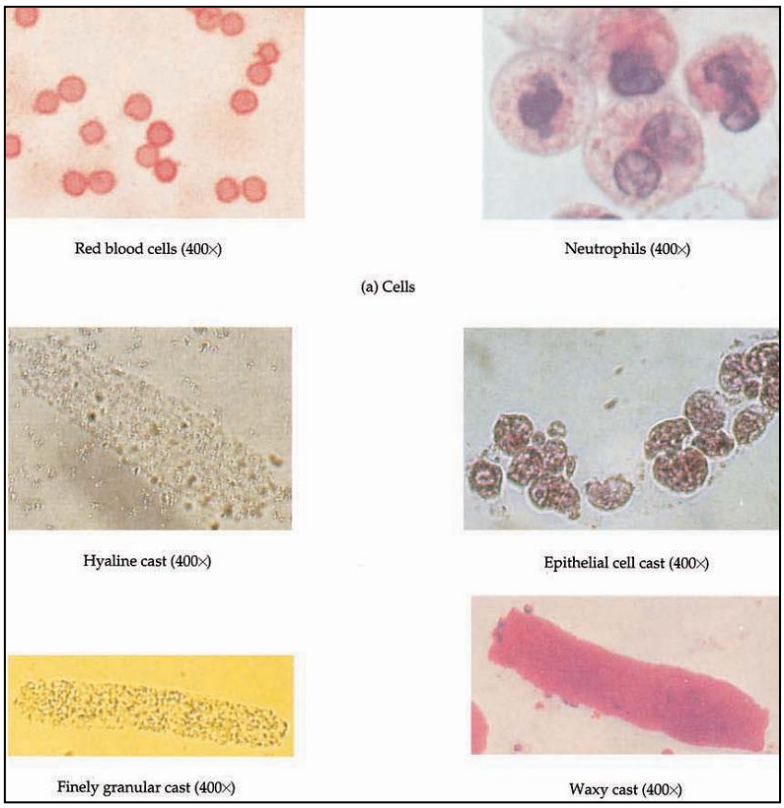
present in the urine.

-Very few or no white or red blood cells or casts are seen.

-A few crystals may normally be seen.

b. Unhealthy

-The above mentioned materials may be seen, according to the cause of the disease.



Crystals	Characteristics of Formation	Appearance	Diagnostic Utility
Uric Acid	Formation promoted by acidic urine		Seen in tumor lysis syndrome
Calcium phosphate	Formation promoted by alkaline urine		Not suggestive of any specific systemic disease
Magnesium ammonium phosphate (a.k.a. struvite or "triple phosphate")	Formation promoted by alkaline urine		Seen in UTIs by urease-producing organisms (e.g. *Proteus*, *Klebsiella*)
Calcium oxalate dihydrate	Formation is largely independent of urine pH		Not suggestive of any specific systemic disease
Calcium oxalate monohydrate	Formation is largely independent of urine pH		Seen in ethylene glycol ingestion
Cystine	Formation promoted by acidic urine		Diagnostic of cystinuria

9.6. Results

-What were the main elements that found in the examined urine? Why?

-Have you seen RBC in the tested sample? What are the sources of RBC in the urine?

-Can you see bacteria in the urine without staining? How?

9.7. Review questions

- Is it necessary for normal individual to check for body fluid every 6 months?

-What are the diseases that leading to hematuria and hemoglobin urea?

-What are the causes of crystals in the urine? Do you believe it is more higher in female urine than male?

-Why sometime we need to collect CSF? Explain?

Experiment 10: Urinalysis (Physical characteristics)

10.1. Aims

1. General evaluation of health .

2. Diagnosis of disorders of the kidneys and urinary tract.

3. Diagnosis of systemic diseases that affect kidney function.

4. Monitoring of patients with diabetes.

5. Screening for drug use/abuse.

10.2. Background

Urinalysis It is ordered widely and routinely to detect any abnormalities that require follow up. Often, substances such as protein or glucose will begin to appear in the urine before people are aware that they may have a problem.

10.2.1. Specimen Collection

1. A specimen of urine may be collected at any time for routine tests; urine voided within 3 hr after meals, however, may contain abnormal constituents. For this reason the first voiding in the morning is preferred.

2. Collect a midstream sample of urine in a sterile container. A midstream sample is essential to avoid contamination from the external genitalia, and to avoid the presence of pus cells and bacteria that are normally found in the urethra.

3.If not examined immediately, the specimen should be refrigerated to prevent unnecessary bacterial growth.

4.Before testing alwaysmix urine by swirling, inverting the container, or stirring with a wooden swab stick.

10.2.2. Sample Information

URINALYSIS

Name: _____	Date: _____
Appearance: _____	Blood: _____
Color: _____	Bilirubin: _____
Protein: _____	Ketones: _____
Spec Grav: _____	Glucose: _____
Sediment: _____	Urobilinogen: _____
Bacteria: _____	RBC: _____
WBC: _____	Crystals: _____
Casts: _____	Epithelium: _____
pH: _____	Notes: _____

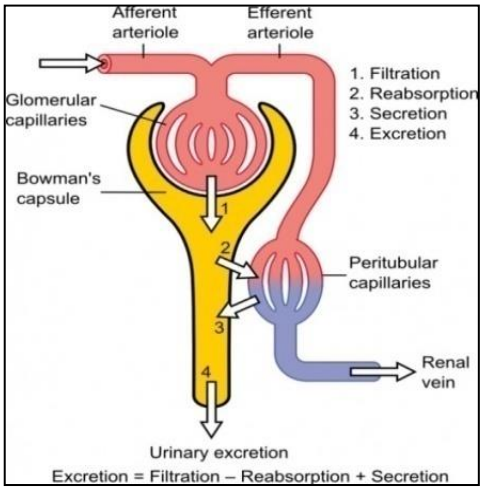
10.2.3. Composition of Normal Urine

-Normal urine contains 90-95 % water and about 5-10% of solid which may be organic (urea, creatineane, uric acid,……) or inorganic (ammonia, sodium, potassium, calcium, phosphates, sulphates,……..) in nature.

10.2.4. Factors Affecting Urine Composition

1. Glomerular filtration.

2. Tubular reabsorption.



10.3. Tubular secretion.

10.4. Materials and Methods

Physical Examination of Urine

1. Colour

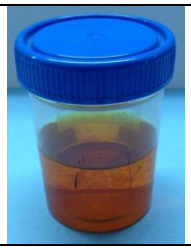
-Freshly excreted urine is colorless to straw colored.

-The normal color of urine is due to the presence of pigment **urochrome.**

-Trances of other substances, such as uroerythrin,urobilin, uroporphyrin and coproporphyrinsalso contribute to the color of urine.

-Variation in Urinary Color

1. Physiological Variation

No.	Color	Interpretation
1	Dark yellow	• Concentrated urine • Mild dehydration • Vitamin B complex therapy 
2	Orange	Medication
3	Pinkish	Excessive beet root or dragon fruit intake

2. Pathological Variations

No.	Color	Interpretation
1	Deep yellow	Jaundice
2	Reddish	Haematuria
3	Brownish	Hemoglobinuria, myoglobinuria and porphyrias
4	Brown to black	Alkaptonuria (lack of an enzyme called homogentisic dioxygenase)
5	Cloudy	Pus cells and bacteria in infected cells
6	Smoky	Red blood cells
7	Black	Iron therapy
8	Pinkish brown	Presence of urobilin – Hemolytic anemias
9	Milky white	Chyluria(Presence of fat globules)

2.Transparency

A fresh urine sample should be clear. Cloudy urine may be due to substances such as mucin, phosphates, urates, fat, pus, mucus, microbes, crystals, and epithelial cells.

3. Volume

-Normal volume is 800-2,500 ml/day with an average of 1500 ml/day.

-Approximately 500 ml/day is the minimum volume of urine needed in normal health to remove waste products.

-The volume of urine is affected by fluid intake and loss, type of diet, cardio-vascular status and renal functions.

-Variation in the Volume of Urine Excreted

A. Polyuria

-It implies an increased volume of urine excreted per day, generally volume of urine exceeding 2,500 ml/day is termed as Polyuria.

-Conditions causing polyuria are diabetes mellitus, diabetes insipidus, diuretics, and alcohol.

B. Oliguria

-Volume of urine less than 500 ml/day is termed oliguria.

-Conditions causing oliguriaare fever, diarrhea, severe edema, acute nephritis, cardiac failure and hypertension.

C. Anuria

-Complete cessation of urine or volume of excreted urine less than 100 ml/day.

-Conditions causing anuriaare acute tubular necrosis, blood transfusion reaction, surgical shock, bilateral renal stones, and sulphonamide therapy.

4. Specific Gravity (sp. gr.)

-It indicates the concentrating ability of the kidneys.

-The normal urinary specific gravity ranges between 1.016-1.025, the average being 1.020.

-The specific gravity is affected by volume of urine excreted and the amount of solids present in the urine.

-Variation in sp. gr. of Urine

A. Low specific gravity- 1.016 or less.

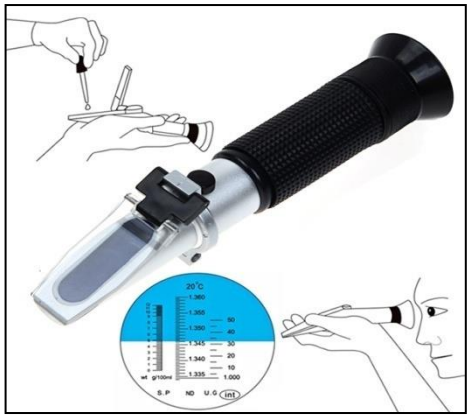
-Conditions causing low specific gravity of urine are polydipsia (abnormally great thirst), diabetes insipidus (ADH or vasopressin deficiency), glomerulonephritis and pyelonephritis.

B. High specific gravity- 1.025 or more

-Conditions causing high specific gravity of urine are severe dehydration, nephrotic syndrome (due to proteinuria), diabetes mellitus (due to glycosuria), adrenal insufficiency (excess of sodium in urine), congestive heart failure, hepatic diseases and extra renal water losses (fever, vomiting and diarrhea).

-Measurement of sp. gr.

-The specific gravity is measured by **Refractometer.**



-Clean the instrument (both the daylight plate and the top of the prism) using a soft, damp cloth before and after use.

- Place 2-3 drops of the urine sample on top of the prism and close the daylight plate.

-Hold the refractometer in the direction of a natural light source and look into the eyepiece.

-You will see a circular field with graduations down the center. You may have to focus the eyepiece to clearly see the graduations and take your reading.

5. Odor

Normal urine has an aromatic odor, although it is affected by many things including sex hormones (males generally have a stronger urine odor).

-Variation in the Odor of the Urine

- **Ammoniacal Odor:** On keeping sample for a long time.
- **Acetone like Odor:** Diabetes mellitus or starvation.
- **Foul smell:** bacterial infections.

6. pH

Normal urine is acidic and the pH ranges between 4.5-8.0 with a mean of 6.0 in 24 hours.

-Variation of Urinary pH

A. Acidic urine

1. Physiologically may be due to a protein rich diet and heavy exercise.

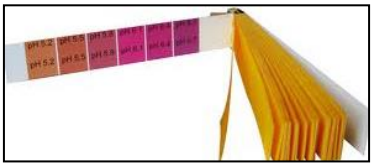
2. Pathologically , It is found in conditions of acidosis, such as diabetic ketoacidosis, respiratory acidosis, and high fever (break down of tissue proteins).

B. Alkaline pH

1. Physiologicallymay be due to heavy meals, diet rich in citrus fruits and excessive intake of milk and antacids.

2. Pathologically may be due to urinary tract infections.

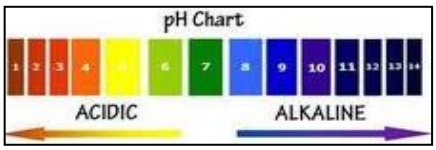
-Measurement of Urinary pH



Urinary pH is measured by:

1. pH papers.

2. Litmus papers.



10.5. Results

-Have you collected urine sample aseptically? How?

-How did you performed Sp.gr. for the urine?

-How many colors of the urine did you have for today? What made this color variation?

10.6. Review questions

-What is the benefit of pH measuring of the urine?

-Can we use pH meter instead of fast measuring papers? Which one is more accurate? Why?

-What is the relationship between the high specific gravity and dehydration?

-Do you think season has physical effect on urine content?

-Why on keeping urine for log time the odor turned to ammonic?

-What is the cause for milky color?

-What shoud be the urine of pregnant women? Why?

Experiment 11: Urinalysis (Chemical characteristics)

11.1. Aims

To determine the abnormal contents of the urine which might be due to renal or systemic disease.

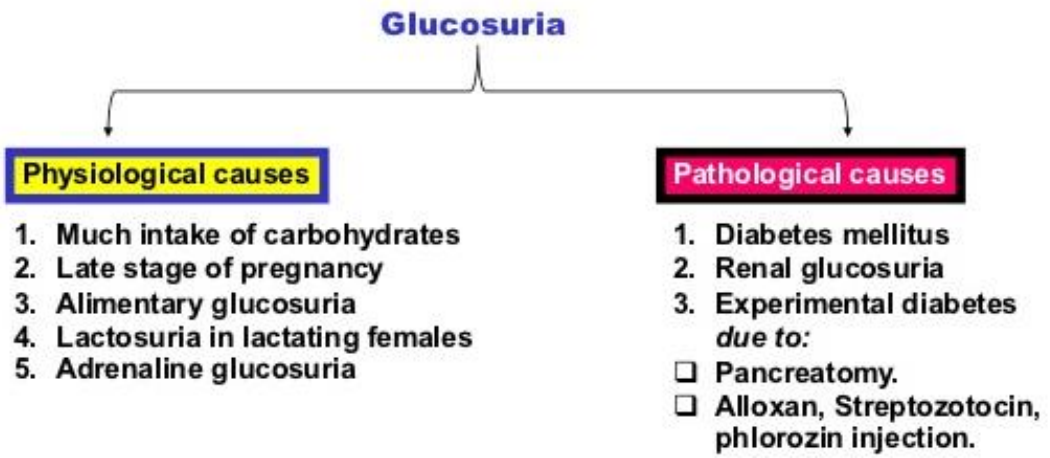
11.2. Background

To perform the chemical examination, most clinical laboratories use commercially prepared test strips. These are narrow plastic strips that hold small squares of paper called test pads, arranged in a row. The test pads have chemicals impregnated into them. When a strip is briefly, but completely, dipped into urine, the test pads absorb the urine and a chemical reaction changes the color of the pad within seconds to minutes.

11.2.1. Abnormal Components of Urine

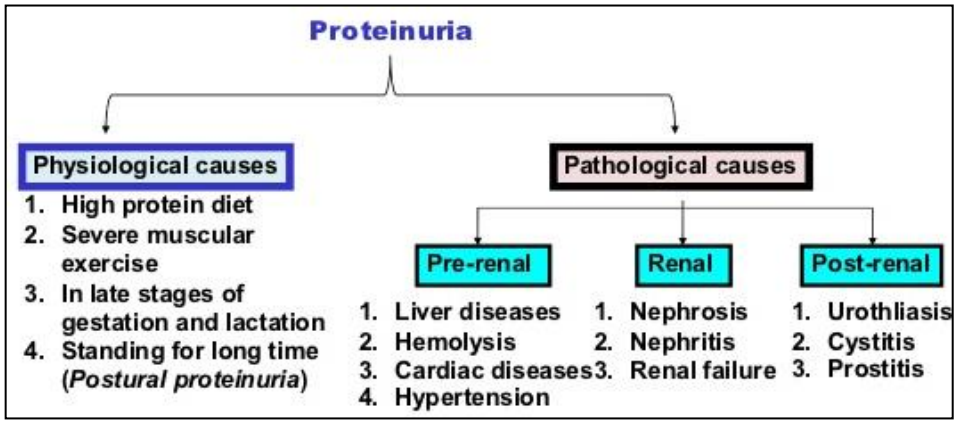
1. Sugars

Normally glucose level is less than 0.1 g/day in urine.



2. Proteins

Normal protein level in urine is less than 30 mg/L.



3. Ketone Bodies

Normal level in urine is less than 18mg/day.

Ketonuria: *its causes are:*

1. Uncontrolled diabetes mellitus
2. Renal glucosuria (diabetes innocence).
3. Much fat intake
4. Starvation for long time
5. Low dietary carbohydrates

4. Billirubin

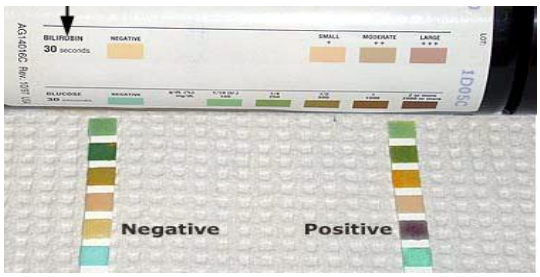
It is mainly present in the patients with obstructive jaundice.

5. Nitrites

Urinary tract bacterial infections are often associated with the presence of nitrites in the urine.These bacteria put out nitrites as a metabolic waste product.

6. Urobilinogen

When the liver is stressed, often extra bilirubin is put out into the intestine. Then, bacteria in the gut often metabolize the bilirubin into urobilinogen which ends up getting into the blood and then into the urine.



11.3. Materials and Methods

TEST STRIPS

-It is composed of plastic strips to which are attached paper squares impregnated with various reagents for pH, protein, glucose, ketones, bilirubin, and blood (hemoglobin), ……

-These strips display a color reaction when dipped into urine with any abnormal constituents.

- Each indicator is an enzymatic test, so timing is CRUCIAL.

11.4. Method

1. Dip the test strip into a urine sample for a limited period of time (almost 1 second) according to the recommendation from the manufacturing company.

2.being sure that all reagents on the strip are immersed.

3.Remove any excess urine from the strip by drawing the edge of the strip along the rim of the container holding your urine sample.

4.After the appropriate time, as indicated on the teststrip vial, hold the strip close to the color blocks on the vial.

5.Make sure that the strip blocks are properly lined up with the color chart on the vial.

-Better to hold the strip horizontally so that reactants from one pad don't run into and taint the reactions going on in another pad.

6. Compare the result, read and record.

11.5. Results

-Which test you have used to determine protein in urine?

-Could you used the same trip twice for 2 different test? Why?

-Is it possible by using this rapid test to make decision about chemical abnormalities of the urine?

11.6. Review question

-In the case of gout disease which element is commonly found in the urine? Why?

-Is there any alternative method to stip test? Which one is more accurate?

-In case of glucosurea, which element in the urine is also affected?

-Which one is more dangerous: proteinurea or ketonurea? Why?

-What are the abnormalities of the liver that affects the chemical composition of the urine?

-Is physical or chemical analysis of the urine is more reliable? Why?

-Do you think high water intake affects chemical composition of urine?

Appendix

English Units of Measurement

Fundamental or derived unit	Units and equivalents
Length	12 inches (in.) = 1 foot (ft) = 0.333 yard (yd)
	3 ft = 1 yd
	1760 yd = 1 mile (mi)
	5280 ft = 1 mi
Mass	1 ounce (oz) = 28.35 grams (g); 1 g = 0.0353 oz
	1 pound (lb) = 453 g = 16 oz; 1 kilogram (kg) = 2.205 lb
	1 ton = 2000 lb = 907 kg
Time	1 second (sec) = 1/86,400 of a mean solar day
	1 minute (min) = 60 sec
	1 hour (hr) = 60 min = 3600 sec
	1 day = 24 hr = 1440 min = 86 400 sec
Volume	1 fluid dram (fl dr) = 0.125 fluid ounce (fl oz)
	1 fl oz = 8 fl dr = 0.0625 quart (qt) = 0.008 gallon (gal)
	1 qt = 256 fl dr = 32 fl oz = 2 pints (pt) = 0.25 gal
	1 gal = 4 qt = 128 fl oz = 1024 fl dr

Metric Units of Length and Some English Equivalents

Metric unit	Meaning of prefix	Metric equivalent	English equivalent
1 kilometer (km)	kilo = 1000	1000 m	3280.84 ft or 0.62 mi; 1 mi = 1.61 km
1 hectometer (hm)	hector = 100	100 m	328 ft
1 dekameter (dam)	deka = 10	10 m	32.8 ft
1 meter (m)	Standard unit of length		39.37 in. or 3.28 ft or 1.09 yd
1 decimeter (dm)	deci = $\frac{1}{10}$	0.1 m	3.94 in.
1 centimeter (cm)	centi = $\frac{1}{100}$	0.01 m	0.394 in.; 1 in. = 2.54 cm
1 millimeter (mm)	milli = $\frac{1}{1000}$	0.001 m = $\frac{1}{100}$ cm	0.0394 in.
1 micrometer (μm) [formerly micron (μ)]	micro = $\frac{1}{1,000,000}$	0.0000001 m = $\frac{1}{10,000}$ cm	3.94×10^{-5} in.
1 nanometer (nm) [formerly millimicron (mμ)]	nano = $\frac{1}{1,000,000,000}$	0.0000000001 m = $\frac{1}{10,000,000}$ cm	3.94×10^{-8} in.

Temperature

Unit	K	°F	°C
1 degree Kelvin (K)	1	$\frac{9}{5}$(K) − 459.7	K + 273.16*
1 degree Fahrenheit (°F)	$\frac{5}{9}$(°F) + 255.4	1	$\frac{5}{9}$(°F − 32)
1 degree Celsius (°C)	°C − 273	$\frac{9}{5}$(°C) + 32	1

* Absolute zero (K) = −273.16°C

Volume

Unit	mL	cm^3	qt	oz
1 milliliter (mL)	1	1	1.06×10^{-3}	3.392×10^{-2}
1 cubic centimeter (cm^3)	1	1	1.06×10^{-3}	3.392×10^{-2}
1 quart (qt)	943	943	1	32
1 fluid ounce (fl oz)	29.5	29.5	3.125×10^{-2}	1

References

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2. Connie Allen, Valerie Harper. Essentials of Anatomy and Physiology Laboratory Manual, 5th edition, 2004.
3. Richard Pflanzer. Experimental and Applied Physiology Laboratory Manual, 8th Edition, 2003.