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Laboratory Exercise 1: Histology

In multicellular organisms, cells form tissues. There are four primary tissue types:

1. Epithelium,
2. Connective tissue
3. Muscle
4. Neural tissue.

Organs, formed from primary tissues, function as parts of organ systems. In this exercise, you will study various cells and tissues and you will learn to distinguish between them. Make drawings and notes on the data sheet. In the first lab exam, you will be tested on your ability to identify the tissues and organs you study today.

PRIMARY TISSUES

I. **Epithelium**: closely packed cells, minimal extracellular fluid, lack of blood vessels and nerve endings

1. Simple epithelia (single cell layer)

    a. Squamous epithelia (flattened cells), e.g., inner lining of blood vessels and heart (endothelium), the alveolar walls in the lungs and renal glomeruli and tubules (thing segment of loop of Henle)

    b. Cuboidal epithelia (cells are equal in height and width), e.g., kidney tubules and secretory cells of many glands

    c. Columnar epithelia (cells are greater in height than width), e.g., inner lining of the stomach, intestines, and gall bladder; the secretory cells of many glands

2. Stratified epithelia (two or more cell layers)

    a. Squamous epithelia, e.g., the keratinized and nonkeratinized epithelium of the skin, the lining of the oral cavity and esophagus

    b. Cuboidal epithelia, e.g., ducts of sweat glands

    c. Columnar epithelia, e.g., parts of the larynx and pharynx

II. <u>Connective tissue</u>: maintains the structural integrity of the body; composed of cells and matrix. The matrix is the extracellular component, containing collagen and elastin fibers

1. Cell types:

   a. Fibroblasts: produce fibers and matrix
   large, irregularly shaped, abundant cytoplasm, ovoid nucleus

   b. Fibrocytes: inactive form of fibroblast, can revert to fibroblast
   smaller and thinner than fibroblasts

   c. Lymphocytes: small cells with large nuclei, they enter the connective tissue from the blood and accumulate in the tissue in pathologic conditions

   d. Macrophages: large, irregularly shaped cells, they enter from blood
   phagocytotic activity (scavengers)
   they migrate easily in the connective tissue

   e. Mast cells: large round cells, their cytoplasm is rich in large, bluish round granules which often obscure the nucleus; they enter from blood (in blood they are called basophil granulocytes).

   f. Adipocytes: they contain a large, single lipid droplet; nucleus is oval and compressed against the cell membrane

2. Collagen fibers: they make up most of the fibrous component, generally appear in bundles, they do not branch.

3. Elastin fibers: they branch and thinner than collagen

4. Ground substance: the material in which connective tissue cells and fibers are embedded (except in bone), viscous gel, occupies most of the tissue space

III. <u>Muscle</u>

1. Striated skeletal muscle: composed of muscle fibers surrounded by connective tissue
   - Fibers are collected into fascicles (bundles).
   - Muscle fibers are multinucleated, nuclei are peripherally located under the cell membrane.

2. Striated cardiac muscle:
   - the fibers are shorter than skeletal muscle fibers and branch to join each other forming sheets rather than bundles.
   - specific junction between the muscle fibers: intercalated disks
   - nuclei are centrally located

3. Smooth muscle:
   - the fibers are fusiform, small but relatively long
   - each fiber has a single, centrally located nucleus
   - fibers are arranged in concentric sheets and are tightly packed

IV. <u>Neural tissue</u>: neurons and glia cells

1. Neurons: vary in size and shape
   cell body – axon – dendrite

2. Glia: interstitial supporting cells in the nervous system
   significantly vary in size and shape

ORGANS

1. <u>Blood</u>:

Erythrocytes: the most abundant cell type, no nucleus

Thrombocytes: may not be seen on your slides, require special staining

Lymphocytes: the second most abundant leukocyte type
round-shaped big nucleus, thin rim of cytoplasm around it.

Monocytes: the largest blood cells
round or oval shaped cells, kidney shaped nucleus, gray-blue cytoplasm

Basophil granulocytes: less than 1% of all leukocytes, you have to be patient to find one
one non-segmented nucleus, which is obscured by large basophilic (usually blue) granules

Eosinophil granulocytes: segmented nucleus (2-3 lobes)
cytoplasm: many large spherical granules that stain with eosinophil dyes (typically red)

Neutrophil granulocytes: the most abundant leukocyte type
segmented nucleus (3-5 lobes)
fine granules in the cytoplasm

2. Arteries

The wall has three layers:
- innermost layer (intima): endothelium
- middle layer (media): smooth muscle and elastic and collagenous tissue
- outer layer (adventitia): loose connective tissue

Arterial wall is thicker and more muscular than the wall of veins.

3. Veins

The wall of the typical, medium-sized veins lacks muscle layer and elastic fibers

4. Bone

Calcified connective tissue

Cell types:
- Osteoblasts: synthesize bone matrix
- Osteocytes: when osteoblasts are surrounded by matrix they are called osteocytes; osteocytes have many tiny processes
- Osteoclasts: giant cells, specialized monocytes, resorb calcified bone matrix

Osteocytes are located in narrow spaces in the calcified matrix called *lacunae*. Adjacent lacunae (i.e., adjacent osteocytes) are interconnected by even thinner structures in the matrix called *canaliculi*.

Lacunae form concentric rings called *lamellae*.

In the center of the lamellae the so called Haversian canals run (longitudinally in the long axis of the bone)

Volkmann's canals run transversely to join Haversian canals. These canals contain nerves and blood vessels.

NOTE: Because of the nature of the technique used for the preparation of the slides, most of the cells, connective tissue and blood vessels are no longer present in the slide. Their former location (i.e., lacunae, canaliculi, lamellae, canals), however, can be easily seen.

5. Salivary glands

Tubuloalveolar glands

Two types of secretory cells:
- Mucous cells: cytoplasm appears empty or vacuolated
- Serous cells: pyramidal shape, basophilic cytoplasm

6. Stomach

The wall of the tubular organs of the gastrointestinal tract has four major layers: *mucosa* (the inner lining of the wall), *submucosa*, *muscularis externa* and the outermost cover of the wall the *serosa*.

While the general characteristics of these four layers are similar along the gastrointestinal canal, the fine structure (cell types, thickness, the presence or absence of villi, etc.) of the layers differs among the various segments. Pay extra attention to those differences since this is how you will be able to identify which segment is shown under the microscope.

a. Mucosa

The luminal surface is punctured with minute depressions called "gastric pits"

The epithelium lining is simple columnar epithelium and mucus-secreting cells

Lamina propria (of mucosa): gastric glands located here (simple tubular glands that empty into a gastric pit)

Cell types of the gastric glands:

- *Chief cells*, the most abundant secretory cells, protein secretion, they stain blue due to high RNA content
- *Parietal cells*, the largest gland cells, cytoplasm appears bright red, they secrete acid
- *Mucous neck cells*

b. Submucosa

c. Muscularis externa

d. Serosa

7. <u>Small intestines</u> (duodenum, jejunum and ileum)

a. <u>Mucosa</u> highly folded inner surface of the intestines with finger-like projections, called *villi.* The mucosa itself has three layers:

1. Mucous membrane: the most superficial layer of the mucosa
   - single layer of *columnar epithelial* cells on the surface
   - mucus-secreting *goblet* cells scattered among the columnar cells
   - crypts of *Lieberkühn*: simple tubular glands, open between the villi and extend as deep as the muscularis mucosae

2. Lamina propria: fills the villi and located between the crypts. Contains vessels, nerves, lymphoid tissue.

3. Muscularis mucosae: the deepest layer of the mucosa, a thin smooth muscle layer

b. <u>Submucosa</u>: thick, connective tissue layer under the mucosa with larger blood vessels, nerves.

Only in the duodenum: *Brunner's glands* (tubuloalveolar glands lined with columnar epithelium, they empty into the crypt of Lieberkühn).

c. <u>Muscularis externa</u>: a thick double-layer of smooth muscle; an inner circular and an outer longitudinal muscle layer

Between the two layers there are enteric neurons which form a network called *Auerbach's plexus*

d. <u>Serosa</u>: the outermost layer of the intestinal wall; loose connective tissue with blood vessels and lymphatic vessels, covered by a layer of epithelium.

8. <u>Large intestines</u>

a. <u>Mucosa</u>
Smooth surface, there are no villi, columnar surface epithelium with goblet cells
Intestinal glands (crypts) appear as impressions in the surface of the mucous membrane.

b. <u>Submucosa</u>

c. <u>Muscularis externa</u>
Typical circular layer
The longitudinal layer form three strong flat bands

d. <u>Serosa</u>

9. Liver

Functional unit is the *lobule*: anastomosing series of hepatic cells (hepatic cords) that radiate outward from the central vein; hexagonal shaped

Find veins, arteries and bile ducts associated with the lobules.

10. Pancreas: a mixed endocrine and exocrine organ

Exocrine pancreas: exocrine glands (acini) secrete digestive enzymes.
Find the acinar cells and excretory ducts

Endocrine pancreas: endocrine glands, secrete insulin, glucagon, and somatostatin
Islets of Langerhans: spherical-semispherical clusters of lightly stained secretory cells. No secretory ducts!

11. Lung

- Bronchioles: simple columnar ciliated epithelium on the surface, smooth muscle and elastic and reticular fibers underneath

- Alveoli: terminal part of the airway; alveolar squamous epithelium forms the alveolar wall surrounding the alveolar space; capillaries are closely associated with alveoli.

- Pulmonary blood vessels

12. Kidney

Functional units are the *nephrons*: glomeruli (renal corpuscle) and tubular system (proximal and distal tubules, loop of Henle)

Glomeruli: spherical, densely stained corpuscles, made up of capillaries; the site of plasma filtration

Tubular system: lined with a single layer of epithelial cells; drains plasma filtrate/urine to the collecting duct

13. Cerebral cortex

The nerve cells are arranged in six layers. The largest neurons are the *pyramidal* cells which are especially numerous in the fifth layer.

14. Spinal cord

A butterfly shaped gray matter (cell bodies of neurons) surrounded by white matter (axons bundles of neurons also called neural fibers).

The gray matter has dorsal and ventral horns and, in several segments, a small lateral horn.

15. Thyroid gland

Functional units are the *follicles*: spherical shape, 0.02-0.9 mm diameter, a central core filled with colloidal material (thyroid hormones are stored here) surrounded by simple epithelium of variable height (hormone precursors are secreted by these cells).

16. Adrenal gland

Two main layers:
- yellowish outer *cortex* that forms most of the mass of the gland
- more reddish inner layer, *medulla*, which is completely covered by the cortex (secretes the hormones adrenaline, noradrenaline and dopamine)

Within the cortex, there are three layers:

- Superficial layer: *zona glomerulosa*, cells are arranged in rounded groupings (secretes aldosterone)
- Middle layer: *zona fasciculata*, cells are arranged in columns two cells wide (secretes glucocorticoids)
- Innermost layer: *zona reticularis*, branching, interconnecting columns of cells (secretes glucocorticoids and sex hormones)

17. Pituitary

Two lobes:

a. Adenohypophysis → develops from the oral cavity

Hormones secreted by three basic types of cells:
- acidophilic cells (stain orange-red, they are the largest cells and the most abundant)
- basophilic cells (stain bluish)
- chromophobic cells (poorly staining cells, more abundant than basophilic cells)

b. Neurohypophysis → develops from the brain (neuronal tissue)
   - axons arriving from the hypothalamus
   - pituicytes

18. Lymph nodes

Covered by a connective tissue capsule

a. Cortex: afferent lymphatic vessels enter the lymph node from the cortical surface; they form subcapsular sinuses

   lymph flows around the lymphatic follicles (closely packed aggregates of lymphocytes) and enters the medulla

b. Medulla: rich in medullary sinuses which join and form the efferent lymphatic vessels

DATA SHEET. Lab 1. Histology NAME:_____

1. Squamous epithelium (find stratified squamous epithelium in the esophagus and find simple squamous epithelium in the inner lining of blood vessels, i.e., endometrium, and the lung alveoli; also check frog skin slide for squamous epithelium)

2. Cuboidal epithelium (find it in the kidney tubules)

3. Columnar epithelium (find columnar epithelium in the mucous membrane of stomach and small and large intestines)

4. Skeletal muscle

5. Cardiac muscle

6. Smooth muscle (find it in the muscularis mucosae and muscularis externa layers of the small and large intestines)

7. Blood
   a) Erythrocytes

   b) Lymphocytes

   c) Monocytes

   d) Neutrophil granulocytes

e) Eosinophil granulocytes

f) Basophil granulocytes

8. Arteries and veins

9. Bone

10. Liver

11. Pancreas

12. Kidney

13. Stomach

14. Duodenum

15. Jejunum

16. Ileum

17. Large intestine

18. Spinal cord

19. Cerebral cortex

20. Adrenal gland

21. Thyroid gland

22. Pituitary gland

23. Lung

24. Lymph node

25. Salivary gland

Laboratory Exercise 2: Blood Physiology

PRECAUTIONS FOR HANDLING BLOOD

To minimize the risk of human infection, the blood that you will be using in this exercise is sheep blood from a professional biological supply company. No other blood should be used. Nevertheless, you must treat this blood as potentially infectious. You must wear disposable latex gloves at all times. Wash your hands at the end of lab. When finished, place the reusable pipettes in the disinfectant solution and rinse the hemocytometers in this solution as well. NEVER MOUTH PIPETTE, only pipette bulbs should be used. Place all of the disposable pipettes and glassware in the orange biohazard bag when finished.

PART I. HEMATOCRIT

Hematocrit is the percent volume of the whole blood that is occupied by blood cells. Because over 99% of the cells are erythrocytes, the hematocrit essentially presents the percentage of total blood volume occupied by erythrocytes. The hematocrit in women typically range from 40 to 45%, in men it is slightly higher, between 42 and 47%. Hematocrit below these values usually indicates anemia, whereas hematocrit above these values indicate polycythemia. Determine the hematocrit of the blood sample provided using the following procedure:

1. With Pasteur pipette and bulb, place a few drops of blood in a small test tube.

2. Place the open end of a heparinized capillary tube against these drops, keeping the tube horizontal. Allow blood to enter the tube until it is about half full.

3. Plug the open end with the special clay provided.

4. Place the tube in the centrifuge with the open end facing towards the center.

5. Have your teaching assistant start the centrifuge and spin the tubes for 5 minutes.

6. You should now see two distinct layers in the capillary tube: one red and one clear. The red layer is the (red) blood cells.

7. Place the hematocrit tube into the hematocrit reader and follow the direction written on it to determine the hematocrit value.

PART II. HEMOGLOBIN (HB) DETERMINATION

This test uses a book of special Tallquist blotting papers that determine Hb content by changing color.

1. Remove a strip of paper from the booklet and place a drop of blood on it. Wait a few minutes, but do not let the blood dry.

2. Match the color produced to the closest color listed in the back of the booklet. The numbers listed on this chart are % Hb content.

3. Convert the % Hb value to grams of Hb/100 ml blood. This is done by multiplying the % Hb value by 16.5 (g).

4. Men normally have Hb contents of 13.6-17.2 g/100 ml blood. Women usually have a slightly lower Hb concentration, 11.5-15.5 g/100 ml blood.

PART III. ERYTHROCYTE COUNT

In human blood, the erythrocyte count is 4.5-5.5 million/mm^3. Determine the red blood cell count of the sheep blood using the following procedure:

1. With a disposable pipette, place 0.1 ml of blood into a test tube. Add 10 ml of Hayem solution into the test tube with the blood. Hayem solution is a hypertonic saline solution that causes the erythrocytes to shrink and thus prevents clumping (sludge formation).

2. Place a cover slip onto a hemocytometer and place it on the stage of a microscope. Use only the cover slip provided.

3. Using a clean Pasteur pipette, place a few drops of the blood solution prepared at the junction of the cover slip and the hemocytometer. Allow the blood to be taken up by capillary action. Let the blood settle for 2 min in the hemocytometer.

4. Count the erythrocytes in the small squares of the hemocytometer. Each of the smaller squares here have an area of 1/400 mm^2.

5. Count the number of red blood cells in 10 of these squares. The erythrocytes that lie on the top of the lines can cause counting errors. To avoid counting the cells on the lines twice, do not count the cells that lie on the lines at the right and the bottom side of the squares.

6. Calculate the number of red blood cells per microliter (mm^3) of blood. You have to take into account the
    a. the factor by which the blood was diluted
    b. the volume of the hemocytometer in which the cells were counted (the depth of the counting chamber is 0.1 mm).

PART IV. LEUKOCYTE COUNT

In human blood, the normal leukocyte count is 5,000-9,000/mm^3.

1. With a disposable pipette, place 0.1 ml of blood into a test tube. Add 1 ml of Turk solution into the test tube with the blood. Turk solution causes the hemolysis the erythrocytes and white blood cells and stains the nucleus of the white blood cells.

2. The counting technique is the same as for erythrocytes, except you count the cells in the larger boxes (1/25 mm^2)

3. Calculate the number of leukocytes per microliter (mm^3) of blood.

PART V. THE SIZE DISTRIBUTION OF ERYTHROCYTES (PRICE-JONES CURVE)

The average diameter of the erythrocytes is between 7 and 8 μm. If the average erythrocyte size is larger than 8 μm or smaller than 7 μm, it indicates pathological changes in the erythrocyte formation.

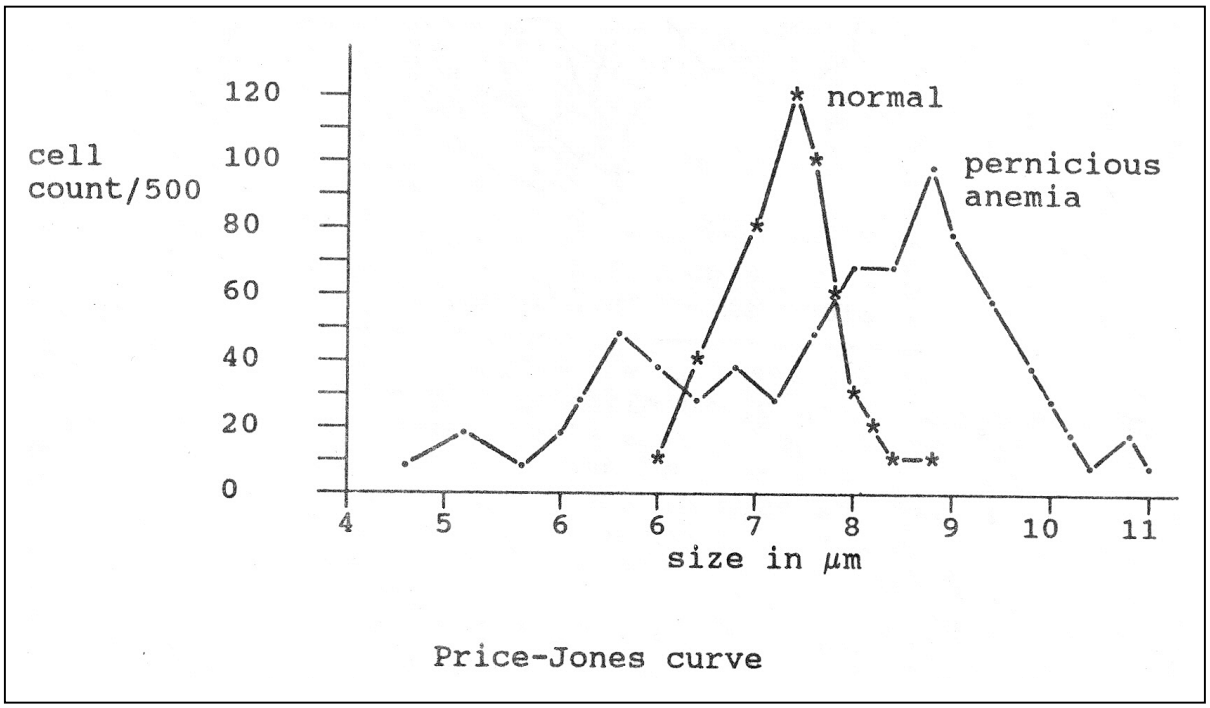
1. Calibrate the scale on the ocular micrometer under immersion enlargement to the edge of a small square in the hemocytometer. Determine the size of one unit on your ocular micrometer.

2. With a disposable pipette, place 0.1 ml of blood into a test tube. Add 20 ml of isotonic NaCl solution (physiologic saline) into the test tube with the blood.

3. Put a drop on a slide and cover it with a cover slip.

4. Measure the diameters of 100 erythrocytes. Count how many of them have a size in the following bands: <5 μm; 5-5.5 μm; 5.6-6.0μm; 6.1-6.5 μm; 6.6-7.0 μm; 7.1-7.5 μm; 7.6-8.0 μm; 8.1-8.5 μm; 8.6-9.0 μm; 9.1-9.5 μm; 9.6-10 μm; >10 μm.

5. Create the frequency-distribution curve. Plot the number of cells (ordinate) for each cell-size band (abscissa) in a coordinate system.



Price-Jones curve

Red blood cell diameters show a Gauss type distribution with a peak between 7 and 8 μm in humans. If the peak falls to a value below 7 μm (the "curve shifted to the left") it indicates ***microcytosis***. Microcytosis is present in one of the most common forms of anemias, in iron deficiency anemia. If the peak falls to a value above 8 μm (the "curve shifted to the right") it indicates ***macrocytosis***. Macrocytosis is present in other forms of anemias, e.g., in pernicious anemia which is due to vitamin B_{12} deficiency. (NOTE: you are using sheep blood for this exercise. Sheep erythrocytes are smaller than human erythrocytes, therefore the peak of the curve will be below 7-8 μm.)

PART VI. TOTAL OXYGEN CARRYING CAPACITY

Each gram of Hb can carry a maximum of 1.34 ml oxygen. Using the Hb concentration of your specimen, calculate the total oxygen carrying capacity of 100 ml sheep blood.

PART VII. MEAN ERYTHROCYTE VOLUME

Use your red blood cell count and hematocrit values to calculate the average volume of sheep red blood cells. Human erythrocytes have a normal volume of ~90 μm^3.

PART VIII. MEASURING THE OSMOTIC RESISTANCE OF RED BLOOD CELLS

A solution that contains the same concentration of osmotically active particles as the intracellular fluid is said to be **isotonic** to the cell. When cells are put into isotonic solution, their size will not change because the net water movement between the cell and the solution is zero. **Hypertonic** solution exerts a greater osmotic pressure than the intracellular fluid, whereas a **hypotonic** solution has lower osmotic pressure than that within the cell. When cells are placed in hypotonic solution, the direction of net water movement will be from the solution into the cell. Erythrocytes can accumulate a certain amount of extra fluid by changing their biconcave shape into a more spherical one (i.e., they "resist" the water flow induced by the hypotonic solution). If the osmolarity of the solution is below a certain level then water flow into the red blood cells will exceed their water-accumulating capacity and the cells will rupture (hemolyse). In this experiment, you will determine the effects of solutions with various osmotic concentration on the integrity of red blood cells.

1. On the table, you will find a container with 5% NaCl solution and a container with distilled water. Make the following dilutions from the 5% NaCl solution: 0.2%, 0.4%, 0.6%, 0.9%, and 2%, 2 ml each in labeled test tubes. Add 2 drops of blood to each of the test tubes (including the 5% solution and another test tube with 2 ml distilled water) and mix gently by inverting the test tube several times. Record the lysis time, i.e., the time when the membranes of all red blood cells ruptured. For the end point of lysis, use the time when you can first see the lines on a piece of ruled notebook paper held behind the test tube. When the RBCs are intact, the solution has an opaque, milky (but red) appearance; the lines behind the test tube appear faint and fuzzy (if visible at all). When the cells are lysed the solution, while still red, will become transparent and a sharp image of the lines can be seen.

2. Place a small drop of each of the following solutions on separate, clean microscope slides: 5% NaCl, 0.9% NaCl, 0.4% NaCl, and distilled water. Add a few red blood cells to each drop by dipping a clean toothpick into a drop of blood and then washing it in the drop of solution on the microscope slide. Gently stir. Add a cover slip and examine as rapidly as possible. Compare the cell size and shape in each solution and explain what happened.

DATA SHEET. Lab 2. Blood **NAME:**_____

Part I. Hematocrit:

Part II. Hemoglobin content:

Part III. Erythrocyte count:

Part IV. Leukocyte count:

Part V. Price-Jones curve

Part VI. Total oxygen carrying capacity

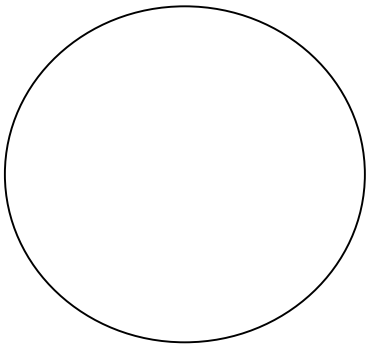
Part VII. Mean erythrocyte volume

Part VIII. Osmotic resistance

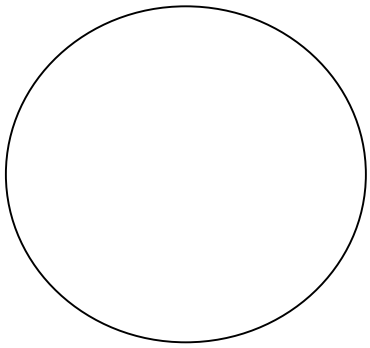
1. Record the lysis time

SOLUTION	Lysis time
distilled water	
0.2% NaCl	
0.4% NaCl	
0.6% NaCl	
2% NaCl	
5% NaCl	

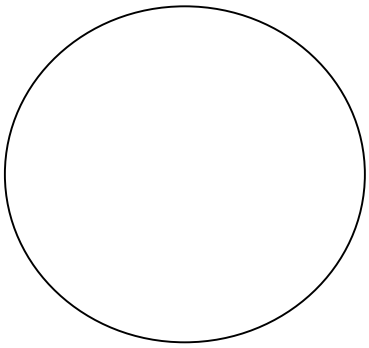
2. Draw the red blood cells you see under the microscope



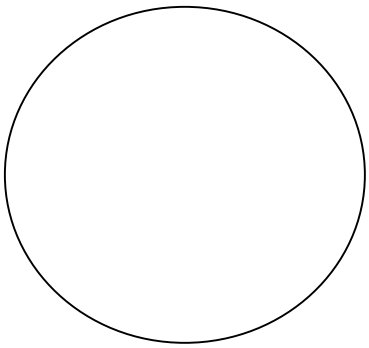
distilled water



0.4% NaCl



0.9% NaCl



5% NaCl

Laboratory Exercise 3: Electrocardiography

BACKGROUND

Sum of the electrical signals from the cardiac muscle as recorded on the surface of the body.

Pattern of the electrical activity depends on the orientation of the electrodes and the electrical activity of the cardiac cells.

CONVENTIONAL ECG ELECTRODE DERIVATIONS: 12 standard leads

ECG is recorded: - bipolar recording: between two points of the body (= bipolar recording)

- unipolar recording: between one point of the body (different electrode) and ground (indifferent electrode)

6 limb leads: I, II, III (bipolar) and aVR, aVL, aVF (unipolar)

6 precordial leads: V_1 - V_6 (unipolar)

THE ECG CURVE

P wave:	atrial depolarization
QRS complex:	ventricular depolarization
T wave:	ventricular repolarization
PR segment:	AV nodal delay
ST segment:	ventricles are contracting and emptying the action potential of ventricular muscle cells in plateau phase
TP interval:	ventricle cells at rest, ventricular filling

EVALUATION OF THE ECG

1. Evaluation of the rhythm:

   a. Regular sinus rhythm (normal)

   b. Irregular rhythm (arrhythmia): it is caused by excitation generated outside the SA node (from an "ectopic focus"). The ectopic focus may be located in the atria (i.e., located "supraventricularly" that is why we call these arrhythmias supraventricular arrhythmias) or in the ventricles. Supraventricular arrhythmias are most often

innocuous, commonly observed in healthy persons while ventricular arrhythmias tend to pose more danger to health.

- Supraventricular arrhythmia, e.g.,
  i. SV paroxysmal tachycardia (stimulus from an irritable, ectopic focus),
  ii. SV extrasystole (premature heartbeat)
  iii. Atrial fibrillation
  iv. Atrial flutter

- Ventricular arrhythmia, e.g.,
  i. Ventricular tachycardia
  ii. Ventricular extrasystole
  iii. Ventricular fibrillation

2. Frequency: - Normal frequency

   - Bradycardia (low heart rate, common among athletes or in response to the stimulation of the vagus nerve, etc.)

   - Tachycardia (increased heart rate, common in response to increased sympathetic activation, increased body temperature, toxic conditions, etc)

3. Orientation of the electrical axis (Einthoven's triangle: vectorial analysis of the QRS complexes)

   Normal orientation of the electrical axis: 59°, points to the left and below horizontal

   Deviations of the electrical axis: - "left axis deviation"
   - "right axis deviation"

   Causes of deviations: - change in the position of the heart
   - left or right ventricle hypertrophy

4. Evaluation of the individual waves/segments/intervals

It reveals various forms of cardiac abnormalities, such as abnormalities in the conduction of the signal in the heart (e.g., atrioventricular block), the necrosis of the cardiac muscle (myocardial infarction), etc., and it may be even diagnostic for general pathological conditions, such as metabolic and electrolyte imbalances (e.g. hyper- or hypokalemia).

EXERCISE

We have six work stations for ECG recordings. You will work in pairs. ECG recordings will be taken from one person per pair. See attached instructions for the description of the exercise. We included a) detailed instructions for today's ECG lab ("Lesson 5, Electrocardiography 1", pages 1-22) and b) general instructions how you can analyze your data once you saved them on the computer ("Measurement Tools", page 77-88).

You will perform the **"L05-ECG-1"** lesson on your computer. Follow the instructions in your attached handout. Briefly, you will have to:

I. Make the following recordings:

1. Attach electrodes to your subject (see page 8 of your instructions)
2. Calibrate your equipment (page 10).
3. You will have to make recordings under three different conditions (page 11-15):
    4. Record from your subject when she/he is in lying on the top of the bench.
    5. Record right after she/he sits up
    6. Record when she/he is breathing deeply.
7. Detach the electrodes and have your subject exercise by walking down the stairs and returning to the third floor. If your subject feels uncomfortable about exercising, this fourth measurement can be left out.
8. Record ECG from your subject after exercise.

II. After the recordings, you will analyze the ECG (page 16-18, also a general description of data analysis on your computer is on pages "77-88"). Setup you display window for optimal viewing of four successive beats. You will measure:

1. Delta T
2. BPM
3. delta amplitude
4. max (amplitude)

Then zoom in on one cardiac cycle and measure the durations of:

5. P wave
6. PR interval
7. QRS interval
8. ST segment
9. T wave

and the amplitudes of:

10. P wave
11. QRS complex
12. T wave

III. Record your findings on the data report sheet (page 19-22).

Laboratory Exercise 4: Blood Pressure and Heart Sounds

PART I. BLOOD PRESSURE

We will measure arterial blood pressure in the brachial artery using the most common, indirect method in the every day practice. Blood pressure could be determined also directly by inserting a cannula, which is connected to a pressure transducer, into the artery. That method is, however, significantly less pleasant for the subject, therefore we will take blood pressure indirectly using the Riva-Rocci sphygmomanometer. This device is an inflatable cuff connected to a gauge that registers pressure within the cuff.

Wrap the cuff around the upper arm. Place the stethoscope over the brachial artery just below the cuff. Inflate the cuff to ~ 170 mmHg, or, if the expected blood pressure is higher than that, then slightly above the expected value. The pressure in the cuff will exceed the arterial pressure in the brachial artery therefore preventing the blood flow in the artery; you will not hear any sound through the stethoscope. Slowly release the air from the cuff. When the pressure in the cuff falls below the systolic pressure then the blood circulation will return in the brachial artery during systole, but not during the entire cardiac cycle. The blood flow will be "turbulent" therefore vibration is produced and each pulse is followed by a short, sharp, knocking sound (Korotkoff sounds). The pressure value in the cuff at the time when the first sound is heard indicates the systolic blood pressure. Continue to release the air from the cuff; from the point when the cuff pressure falls below the diastolic pressure, the artery will be completely open throughout the entire cardiac cycle. The turbulence in the blood flow stops, the flow will be smooth again, no vibrations are generated and the sound over the brachial artery disappears. The cuff pressure value at which the sounds disappear indicates the diastolic blood pressure.

Please, never increase the pressure above 200 mmHg in the cuff, and do not keep the cuff inflated on the arm for an extensive period of time; once it is pumped up to the appropriate value start deflating the cuff right away.

1. Measure the blood pressure in sitting, relaxed subject, then ask the subject to submerge his/her arm in ice cold water for one minute and measure the blood pressure again thereafter.

2. Measure blood pressure in a subject who has been in a reclined position for one minute. Then ask the subject to stand up and measure the blood pressure immediately after he/she has stood up.

3. Measure the blood pressure in sitting, relaxed subject, then asked him/her to walk down to the basement and back up to the lab. Measure the blood pressure immediately after he/she has returned. If you have a medical condition that you feel may be aggravated by the exercise, please inform the instructor.

PART II. HEART SOUNDS

Heart sounds are caused by the **closure of the valves** in the heart. During one cardiac cycle two hearts sounds can be heard:

1. The first is the systolic sound which is caused by the simultaneous closure of the two cuspidal valves.

2. The second sound is the diastolic sound which is due to the simultaneous closure of the two semilunar valves.

Both heart sounds can be heard above the entire heart. However, there is a certain point on the chest where the sound made by a particular valve can be heard the most clearly; this point on the chest is the punctum maximum for the given valve. The punctum maximum does not correspond to the location of the valve itself; it corresponds to the location on the chest where the blood that passed the given valve runs the closest to the chest wall, e.g., the punctum maximum of the mitral valve is above the apex of the heart, because the apex part of the left ventricle lies the closest to the inner surface of the thoracic cavity.

The punctum maximum of the valves

Left semilunar ("aortic") valve:	2nd intercostal space on the *right* side of the sternum.
Right semilunar (pulmonary) valve:	2nd intercostal space on the *left* side of the sternum.
Mitral valve:	above the apex of the heart; 5th intercostal space, ~2 inches from the midline.
Tricuspid valve:	4th intercostal space, on the left side of the sternum.

The significance of auscultation is that the appearance of abnormal heart sounds (called murmurs) give an indication about the function of the valves. For example, stenotic valves give a whistling sound when blood is supposed to flow through them (e.g. stenotic mitral valve whistles during diastole). Insufficient (leaky) valves, on the other hand, give a swishing sound when blood is not supposed to flow though them under normal conditions (but it does if the valve is leaky, e.g., insufficient mitral valve causes murmur during systole).

In the evaluation of heart murmurs it is important therefore to note the timing of the murmur (systolic vs. diastolic), the quality of the sound (whistling vs. swishing), the dynamics of the sounds (crescendo, decrescendo, crescendo-decrescendo, etc.), and, most importantly, the location of the sound (i.e., where is the murmur the loudest, which punctum maximum location?)

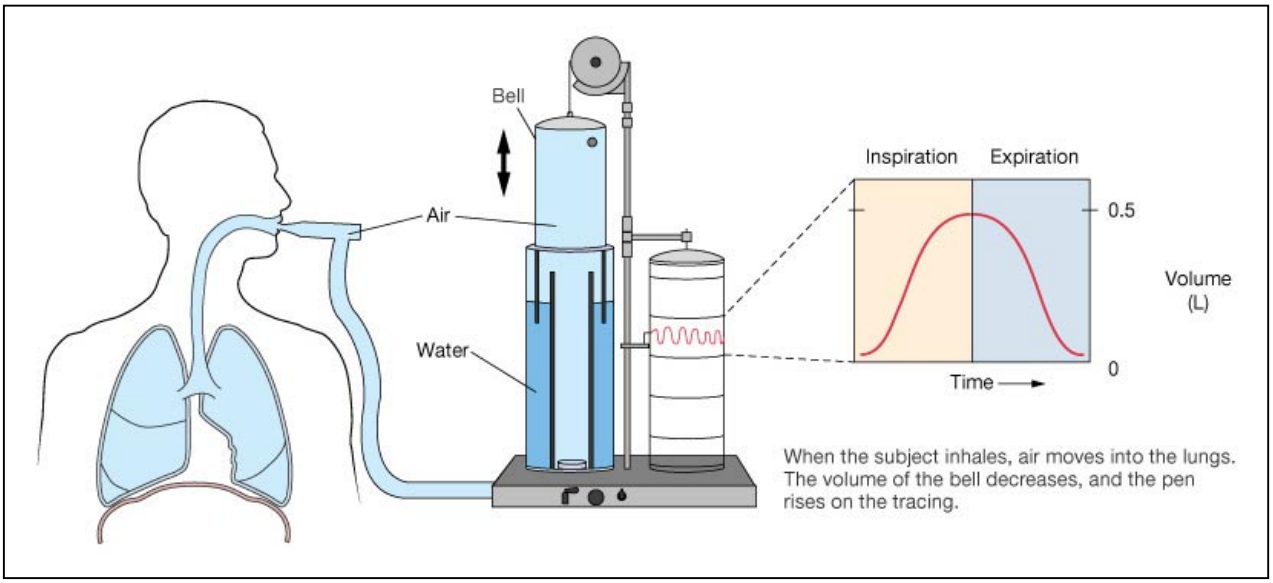
DATA SHEET. Lab 4. Blood pressure NAME:_____

EXERCISE

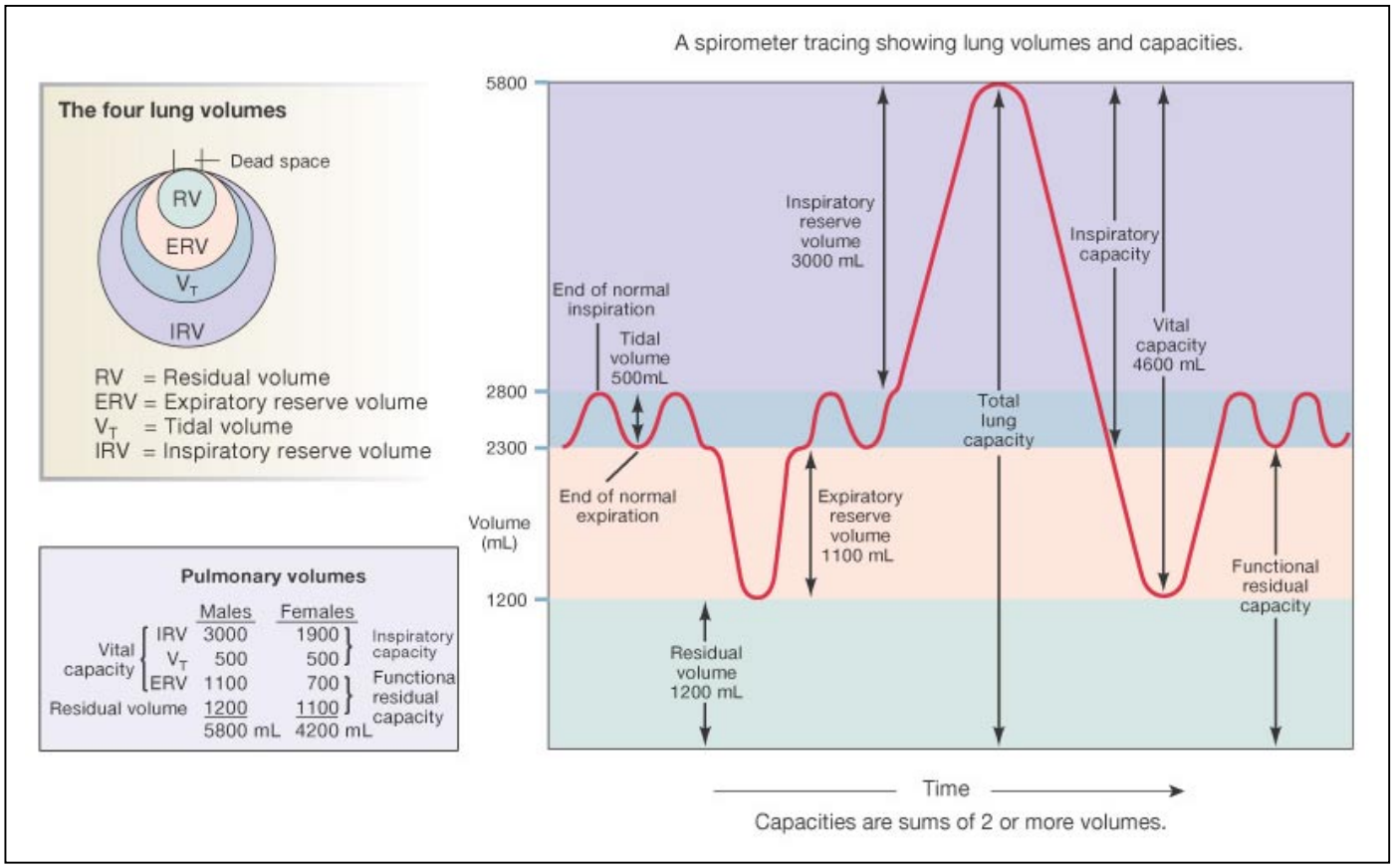
	Systolic pressure	Diastolic pressure
Sitting, relaxed		
Arm in cold water		
Reclined		
Stand-up		
Sitting, relaxed		
After exercise		

Laboratory Exercise 5: Pulmonary Volumes and Capacities (Spirometry)

PART I. BASIC LUNG VOLUMES AND CAPACITIES



Lung volumes and air flow are recorded using a spirometer. The subject inserts a mouthpiece that is attached to an inverted bell filled with air. The volume of the bell and the volume of the subject's respiratory tract create a closed system. When the subject inhales, air moves from the bell into the lungs. The volume of the bell decreases, and the pen rises on the tracing. When the subject exhales, air moves from the lungs back into the bell, and its volume increases. This increase causes the pen to drop on the tracing. The spirometer tracing is called spirogram. You will use a computerized version of spirometer.



You will perform the **"L12 –PUL.1"** and **"L12-PUL.2"** lessons on your computer. Follow the instructions in your attached handout. Briefly, you will have to:

LESSON 12: PULMONARY VOLUMES AND CAPACITIES

I. Make the following recordings:

1. Calibrate your equipment (page 9).
2. You will have to make recordings as follows (page 11-14):
    3. Breath normally for 3 breaths.
    4. Inhale as deeply as you can
    5. Exhale just to the point of normal breathing
    6. Breath normally for 3 breaths
    7. Exhale out completely
    8. Breath normally for 3 breaths

II. After the recordings, you will analyze your data as described on page 15-17.

III. Record your findings on the data report sheet (page 19-22).

LESSON 13: PULMONARY FLOW RATES

I. Make the following recordings:

1. Calibrate your equipment (page 10).
2. You will have to make **FEV** recordings as follows (page 12-16):
    6. Breath normally for 3 cycles.
    7. Inhale as deeply as you can, then hold your breath for just an instant
    8. Exhale as quickly and completely as you can
    9. Breath normally for 3 more cycles
10. Then you continue with MVV recordings as follows (page 16-18):
    11. Breath normally for 5 cycles
    12. Breath quickly and deeply for 12-15 sec
    13. Breath normally again for 5 more cycles

II. After the recordings analyze your data as described on page 19-23.

III. Record your findings on the data report sheet (page 25-28).

IV. Write the following parameters on the blackboard:

- your average TV
- your average VC
- your average FEV1
- your average ERV
- your heights
- your gender
- whether you are a smoker or not

PART II. RESPIRATORY CYCLE

You will perform the **"L08 –Resp-1"** lesson on your computer. Follow the instructions in your attached handout. Briefly, you will have to:

I. Attach the respiratory transducer and the temperature transducer to your subject (page 7-9)

II. Make the following recordings:

1. Calibrate your equipment (page 10).
2. You will have to record from your subject while he/she is (page 11-15):
    4. Breathing normally for about 15 seconds.
    5. Hyperventilating for 30 sec
    6. Recovering from hyperventilation for 30 sec
    7. Holding his/her breath for 30 sec
    8. Recovering for 30 sec after non-breathing
    9. Reading aloud

II. Analyze your data as described on page 16-19.

III. Record your findings on the data report sheet (page 21-24).

PART III. HEYMER TEST OF THE RESPIRATORY RESERVE

Take five deep breaths and then hold your breath for as long as possible on the last. Time how long you can hold your breath.

PART IV. STATISTICAL ANALYSIS OF THE DATA FROM THE ENTIRE CLASS

Using the data that you and your classmates wrote on the blackboard, please perform the following calculations.

1. Calculate the average TV, VC, FEV1, and ERV separately for the groups of smokers and nonsmokers. Also, calculate the average TV, VC, FEV1, and ERV separately for the groups of females and males.

2. The average values within a group (e.g., average VC in the group of smokers) alone do not reliably describe the measured parameter in that group. In addition to the average, another measure needs to be calculated which is called Standard Deviation (SD). Standard Deviation is a measure that indicates the extent to which individual values differ from the mean. In general, the less individual values differ from the mean (i.e., the lower the SD value is) the more reliable your group average is. High SD values usually indicate some sort of sampling error.

   You can calculate the Standard Deviation using the following equation:

$$\text{Standard Deviation} = \sqrt{\text{Sum of (idividual value} - \text{Mean)}^2 \text{ / (Number of values minus one)}}$$

   EXAMPLES:

   For example, if the VC values of five individuals are: 4.4, 4.6, 4.7, 4.3, and 4.5 liters, then the average (mean) is 4.5 liter. The individual values do not differ greatly from the group average; the differences between individual values and group average are: 0.1, 0.1, 0.2, 0.2, and 0.

Individual Values	Individual Value - Mean	(Individual Value - Mean)^2
4.4	-0.1	0.01
4.6	0.1	0.01
4.7	0.2	0.04
4.3	-0.2	0.04
4.5	0	0

   Sum of (individual value - Mean)^2 = 0.01 + 0.01 + 0.04 + 0.04 + 0 = 0.1
   Number of values minus one = 5 -1 = 4
   Standard Deviation = $\sqrt{0.1/4} = \sqrt{0.025} = 0.158$

In another example, if the individual values of five individuals are: 4.4, 4.6, 4.7, 4.3, and 0.4 liters, then the group average (mean) is 3.68 liter. In this case, however, the individual values greatly differ from the group average as shown in the following table:

Individual Values	Individual Value - Mean	(Individual Value - Mean)^2
4.4	0.72	0.518
4.6	0.92	0.846
4.7	1.02	1.04
4.3	0.62	0.41
0.4	-3.28	10.758

Sum of (individual value - Mean)^2 = 0.518 + 0.846 + 1.04 + 0.41 + 10.758 = 13.572
Number of values minus one = 5 -1 = 4

$$\text{Standard Devition} = \sqrt{13.572 / 4} = \sqrt{3.393} = 1.842$$

The relatively high value of SD in the second case suggests that the results are less reliable than in the first case. The high SD is actually due to one individual value which is much lower than the rest of the values in this group. It is very likely that this individual value, 0.4 liter, is due to some sampling error rather than it is a physiologically meaningful value.

Calculate the Standard Deviation of TV, VC, FEV1, and ERV separately for the groups of smokers and nonsmokers. Also, calculate the Standard Deviation for TV, VC, FEV1, and ERV separately for the groups of females and males.

3. Plot the individual VC values (dependent variable on the ordinate; range 2 liters - 7 liters) as a function of body height (independent variable on the abscissa; range 50" - 80") in a coordinate system. Do you see any apparent correlation between heights and VC?

DATA SHEET. Lab 5. Respiration NAME:_____

Part I. Your basic lung volumes and capacities

	First measurement	Second measurement	Third measurement	Average of three measurements
Tidal Volume				
Vital Capacity				
Expiratory R.C.				
FEV1				
FEV2				
FEV3				

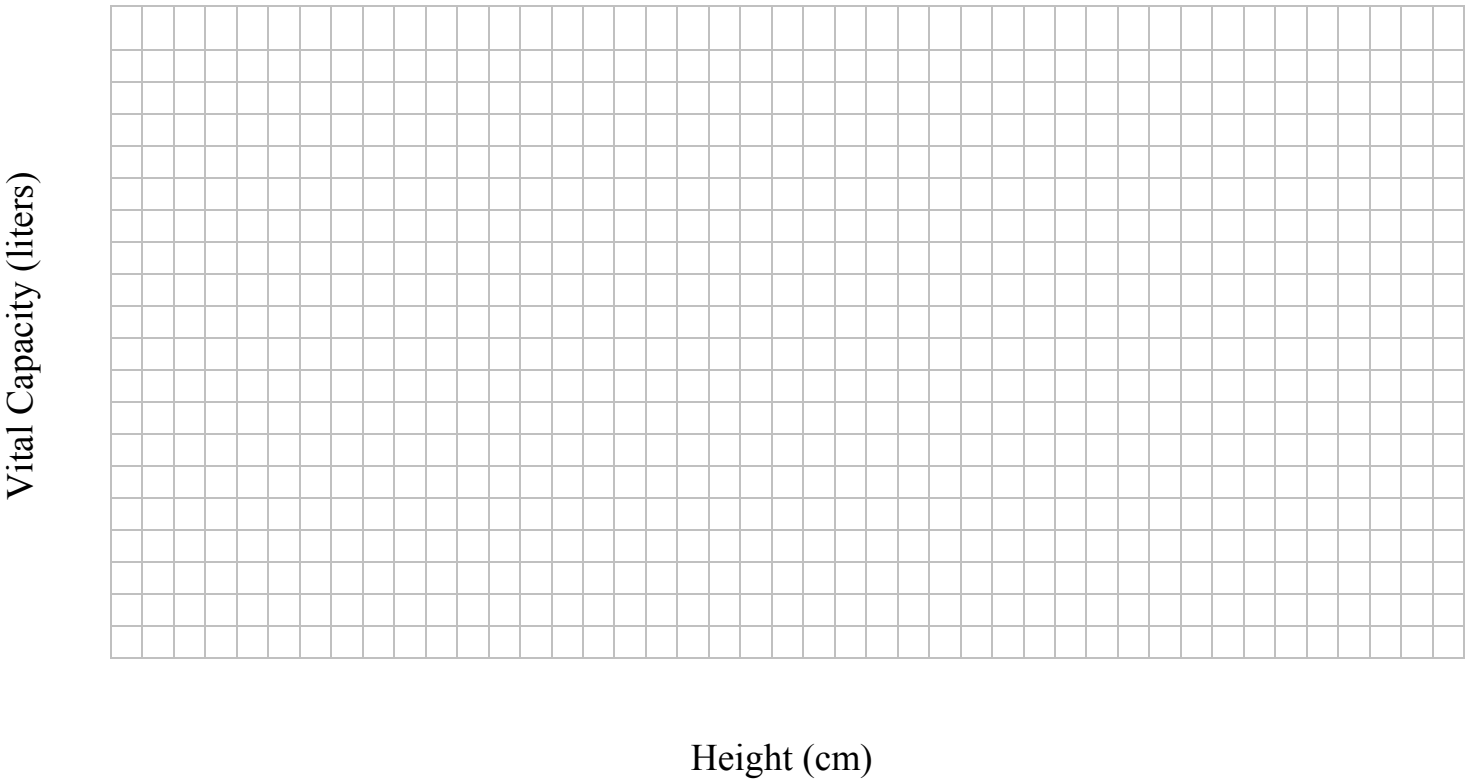
Part II. Heymer test of the respiratory reserve

Part III. Statistical analysis of the data from the entire class

	Tidal V.	Vital C.	Expiratory R.C.	FEV1	Male/ Female	Smoker?	Height
Subject 1							
Subject 2							
Subject 3							
Subject 4							
Subject 5							
Subject 6							
Subject 7							
Subject 8							
Subject 9							
Subject 10							
Subject 11							
Subject 12							
Subject 13							
Subject 14							
Subject 15							
Subject 16							
Subject 17							
Subject 18							
Subject 19							
Subject 20							
Subject 21							
Subject 22							
Subject 23							
Subject 24							
Subject 25							
Subject 26							

	Tidal Volume	Vital Capacity	Expiratory R.C.	FEV1
Nonsmokers, mean				
Nonsmokers, SD				
Smokers, mean				
Smokers, SD				
Females, mean				
Females, SD				
Males, mean				
Males, SD				

Correlation Between Height and Vital Capacity



Laboratory Exercise 6: Urine Analysis

BACKGROUND

Urine normally consists of 95% water and 5% solids (mostly urea and NaCl). Almost all of the substances found in urine are also found in the blood, but at different concentrations. The pH of the urine depends on the quality of the nutrient; usually it is about pH 6. The urine specific gravity depends mainly on the NaCl concentration. When the kidneys are maximally diluting, specific gravity is ~1.001 g/ml. When the kidneys are maximally concentrating, specific gravity can be as high as 1.030 g/ml. Normal urine contains no glucose, amino acids, ketones or proteins. The appearance of glucose together with ketones indicates diabetes mellitus. Protein and blood in the urine is a sign of nephritis, a disease in which glomeruli are damaged and plasma proteins and erythrocytes leak into the kidney tubules. Also, protein and blood are commonly appear in urine in innocuous infections of the lower urinary tract, such as cystitis (the inflammation of the urinary bladder).

One of the kidney's main function is to maintain the osmolarity of the extracellular fluids at 300 mosmol/liter by regulating the excretion of water and salts. You will perform an experiment that demonstrates the regulation of osmolarity. We will present the kidney with excess water or salt load (i.e., you will drink large amount water or eat salty pretzels) and record the salt concentration and volume of urine produced.

SYMPTOMS ASSOCIATED WITH THE DISEASES OF THE URINARY SYSTEM

Polyuria: > 2500 ml urine/day is voided. May be caused by increased water intake (e.g., compulsive water drinking), osmotic diuresis (e.g., glucosuria from uncontrolled diabetes mellitus), decreased vasopressin release in certain hypothalamic or posterior pituitary diseases, or decreased renal tubular response to ADH.

Oliguria: < 500 ml urine/day. Caused by decreased renal blood flow, the obstruction of the urethra or bladder, or a primary disease of the kidneys.

Anuria: < 100 ml urine/day voided. Signals renal failure, the end stage of renal insufficiency.

Nocturia: Voiding during the night. Most often it occurs without disease, e.g., due to excessive drinking in the late evening.

Enuresis: Bed-wetting. It is physiologic during the first 2 or 3 yr of life but later becomes an increasing problem. It may be caused by delayed neuromuscular maturation of the lower urinary tract or organic disease, e.g., infection or distal urethral stenosis in girls.

Dysuria: Painful urination. It suggests irritation or inflammation in the bladder neck or urethra, usually due to bacterial infection.

Obstructive symptoms (hesitancy, straining, decrease in force and caliber of the urinary stream, terminal dribbling) are commonly due to obstruction of urethra. In men, such obstruction is usually due to the enlargement of the prostate.

Urinary incontinence: an uncontrollable loss of urine, usually caused by bladder dysfunction or spinal cord injuries. Also, incontinence in response to mild physical stress (e.g., coughing, laughing, running, lifting) is not uncommon in healthy women. This may be due to urethral atrophy (from a lack of estrogen or as a normal consequence of aging) or stretching of the pelvic floor muscles during childbirth.

LABORATORY FINDINGS ASSOCIATED WITH THE DISEASES OF THE URINARY SYSTEM

Proteinuria: the presence of proteins, most commonly albumin, in the urine. The major mechanisms producing proteinuria are elevated plasma concentrations of normal or abnormal proteins; increased tubular cell secretion; decreased tubular resorption of normally filtered proteins; and an increase of filtered proteins caused by altered glomerular capillary permeability. *Exercise proteinuria* sometimes occurs in joggers, marathon runners, and boxers

Glucosuria: The presence of glucose in urine. The most common cause of glucosuria is diabetic hyperglycemia with normal renal glucose transport.

Ketonuria: Acetoacetic acid, acetone, and β-hydroxybutyric acid are excreted in the urine (the traditional common name for these molecules is "ketone bodies" in the parlance of medicine, hence the name of the phenomenon). Typically, it is not a sign of a disease of the urinary tract, rather, it accompanies certain metabolic abnormalities, such as starvation, diabetes mellitus, and ethanol intoxication.

Hematuria: The presence of red blood cells in urine. Macroscopic hematuria: significant amounts of blood stain urine reddish; visible for the unaided eye. Microscopic hematuria: the color of urine appears normal but microscopic examination or other laboratory tests reveal the presence of blood. The dipstick reagent is sensitive to free hemoglobin and myoglobin. A positive test in the absence of RBCs on microscopic examination suggests hemoglobinuria or myoglobinuria.

Osmolality: The total concentration of solutes in urine, expressed as mOsm/kg (mmol/kg) of urine water. Normal urine osmolality is 100 to 1200 mOsm/kg depending on the circulating level of vasopressin and the rate of urinary solute excretion. The loss of urinary concentrating capacity is a sensitive test of renal dysfunction.

Specific gravity of the urine is measured by a urinometer. Although the correlation with osmolality is not linear, in the everyday clinical use it gives a reasonable indication of the amount of solutes in urine.

Urinary pH: Measured by a dipstick impregnated with various dyes that change color when the pH is 5 to 9.

MEASUREMENT OF RENAL FUNCTION

Renal function tests are useful in evaluating the severity of kidney disease and in monitoring its progress.

Serum creatinine levels can be used as an index of renal function because creatinine production and excretion are reasonably constant. Serum concentration of creatinine varies inversely with the GFR and therefore is a useful index of it.

Creatinine clearance The creatinine clearance (Cl_{creat}) can be calculated from the serum creatinine concentration.

BUN (blood urea nitrogen). The condition when BUN is elevated is called ***azotemia***.

Tests of **renal concentrating capacity** are simple and diagnostically helpful. A loss of concentrating capacity in the presence of adequate vasopressin stimulation is associated with tubulointerstitial disease. Renal concentrating capacity is best tested by water deprivation and by the response to exogenous vasopressin.

Measurement of the **renal plasma flow** is no more useful clinically than the GFR but is more difficult and costly.

SAFETY RULES

1) You must wear latex gloves.

2) Each student will analyze his/her own urine, you are not to handle another person's urine.

3) Urine should be collected and held only in the disposable containers.

4) Once you finished analyzing a specimen, it should be flushed down the designated drain with ample amounts of water. The disposable container must be placed in the biohazard bag next to the sink.

5) At the end of the lab, remove your gloves and place them in the biohazard bag. Wash your hands in the other, clean sink.

6) The chemicals used for analyzing urine are toxic. Avoid contact with your eyes, skin, and mouth. Do not inhale their vapors.

PART I: THE EFFECTS OF WATER AND/OR SALT LOAD ON URINE FORMATION

The class has been divided into four groups:

Group I: control group, subjects do not eat or drink.
Group II: drink 500 ml of water
Group III: drink 500 ml water and eat one bag of salted pretzels
Group IV: drink 50 ml water and eat one bag of salted pretzels

1. Take urine sample before eating/drinking (time zero) and the collect urine samples 30, 60, and 90 min after eating/drinking.

2. Measure the volume that you produced at each time point.

3. Discard the samples except for ~25 ml. Bring this amount back to the lab for analysis

4. Measure: pH, specific gravity, glucose, protein, NaCl content of the urine. Calculate the ***total grams*** of NaCl in the urine collected at each 30-min period. Record all your results on the data sheet as well as on the blackboard.

5. Calculate group averages from the data on the blackboard. Plot the "volume", "specific gravity" and "NaCl content" group results on a graph.

PART II: ANALYSIS OF SYNTHETIC URINE

NOTE: Even though we use synthetic urine, all of the above safety procedures must be followed.

For this exercise, you will be working in pairs. Each pair should have 4 urine samples: A, B, C, and D. Your job is to determine which of these samples has come from a "healthy" patient. You will analyze the samples for:

1. Specific gravity: Use ***urinometer***. Fill the cylinder to about 1 inch from the top with urine. Slowly insert the urinometer into the cylinder. Do not wet the stem above the water line or you will get an inaccurate reading. Give the float a gentle swirl, and read the specific gravity from the graduated marks on the stem as it comes to rest. Do not accept a reading if the float is touching the side of the cylinder. Rinse the urinometer with tap water when you are finished. Measure the temperature of the urine immediately. The urinometer is calibrated to give a correct reading only if the urine is 15 ^{o}C. If your sample is at a different temperature, you will need to correct the specific gravity by adding 0.001 for each 3 ^{o}C above, or subtracting 0.001 for each 3 ^{o}C below the temperature 15 ^{o}C.

2. Glucose: place 2 ml of urine into a disposable test tube with a pipette. Add 2 ml of Benedict's solution and place the test tube into the hot water bath for 5 min. After the incubation, note the color of the solution and record the glucose content using the following scale:

Color	Amount of Glucose (Arbitrary Units)
Blue	none
Green	1
Yellow	2
Orange	3
Red	4

3. NaCl: Place ten drops of urine into a test tube. Add 1 drop of 20% potassium chromate. Add 2.9% silver nitrate to this mixture, one drop at a time, shaking the mixture continuously while silver nitrate is being added. Count the number of drops needed to change the color of this mixture to a brown color. Each drop of silver nitrate required corresponds to 1 mg/ml NaCl concentration in the urine.

4. pH, proteins, ketones, blood: Use Labstix reagent strips. Examine the strip carefully before making the test so you will know which portions to read first. When ready, dip the reagent portion of the strip into the well-mixed urine specimen, wetting all the reagents completely. Wipe the excess urine off on the lip of the urine-analysis bottle. After exactly 15 seconds read the ketone portion against the appropriate color standard. Exactly 15 seconds later read the blood portion. The pH and protein portions may be read after this at your leisure, because time is not so critical with these two.

DATA SHEET. Lab 6. Urine analysis **NAME:**_____

Part 1: The effects of water and salt load on urine formation

1. Results of the analysis of your own urine.

Subject number: _____ Group: _____

Time	Volume (ml)	Specific Gravity (g/ml)	pH	NaCl (mg/ml)	Glucose	Protein
30 min						
60 min						
90 min						

2. Statistical analysis of the group results

Group 1: Control

Time	Volume (ml)	Specific Gravity (g/ml)	pH	NaCl (mg/ml)	Glucose	Protein
30 min						
average ± SD					X	X
60 min						
average ± SD					X	X
90 min						
average ± SD					X	X

Group 2: 500 ml of water

Time	Volume (ml)	Specific Gravity (g/ml)	pH	NaCl (mg/ml)	Glucose	Protein
30 min						
average ± SD					X	X
60 min						
average ± SD					X	X
90 min						
average ± SD					X	X

Group 3: 500 ml water and one bag of salted pretzels

Time	Volume (ml)	Specific Gravity (g/ml)	pH	NaCl (mg/ml)	Glucose	Protein
30 min						
average ± SD					X	X
60 min						
average ± SD					X	X
90 min						
average ± SD					X	X

Group 4: 50 ml water and one bag of salted pretzels

Time	Volume (ml)	Specific Gravity (g/ml)	pH	NaCl (mg/ml)	Glucose	Protein
30 min						
average ± SD					X	X
60 min						
average ± SD					X	X
90 min						
average ± SD					X	X

Volume

Specific gravity

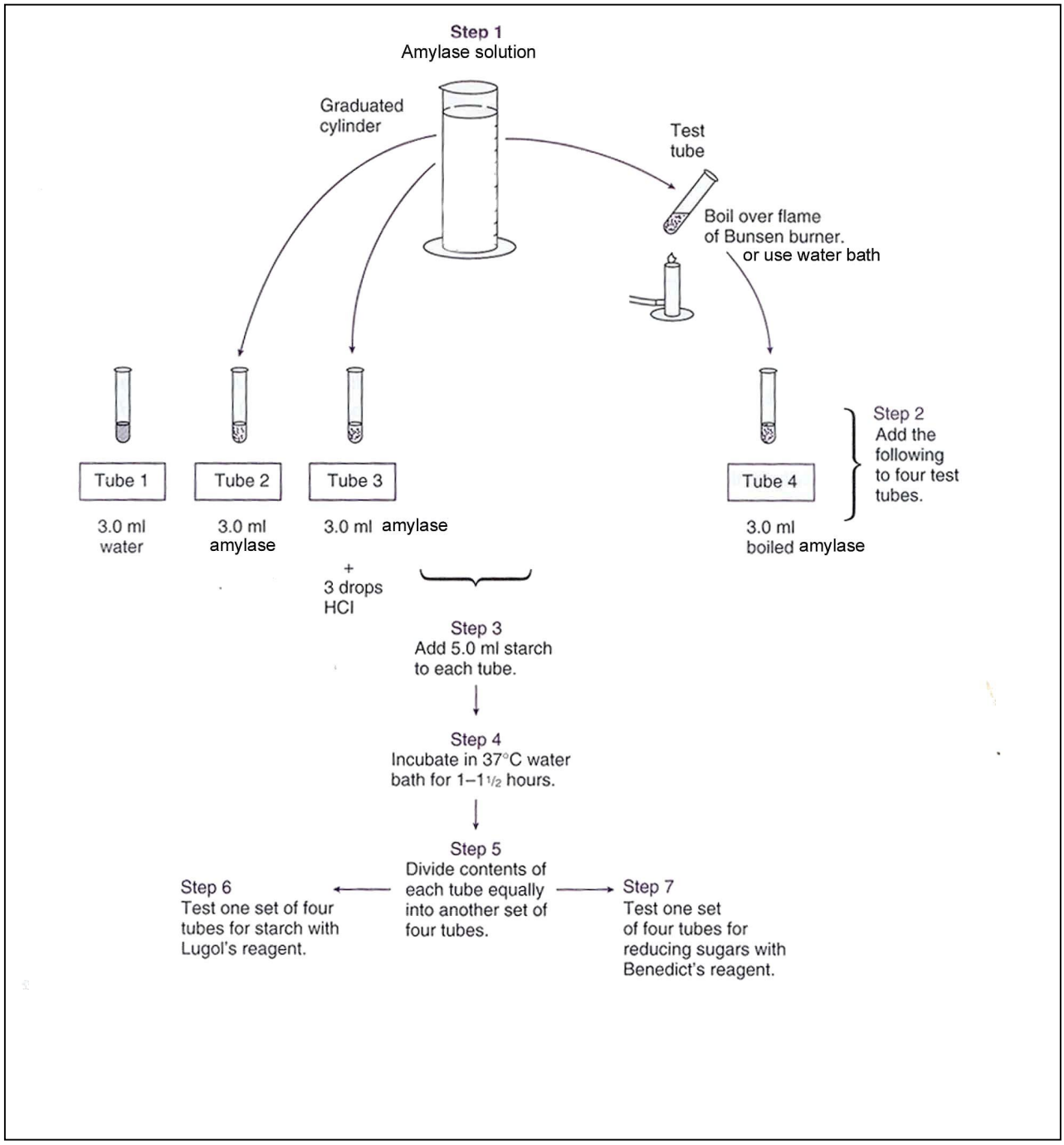
NaCl content

Part 2: <u>The analysis of synthetic urine</u>

Sample	Specific Gravity (g/ml)	Glucose	NaCl (mg/ml)	pH	Protein	Ketones	Blood	Your conclusion
A								
B								
C								
D								

Laboratory Exercise 7: Digestion

PART I: THE EFFECTS OF pH AND TEMPERATURE ON THE ACTIVITY OF AMYLASE



1. Label 4 test tubes

2. Add 3 ml distilled water to tube 1
3. Add 3 ml amylase solution to tubes 2 and 3
4. Add 3 drops of HCl to tube 3
5. Boil amylase solution and add 3 ml of this boiled amylase to tube 4

6. Add 5 ml starch solution to each of the four tubes

7. Incubate all tubes for 1 h in a 37 Celsius water bath

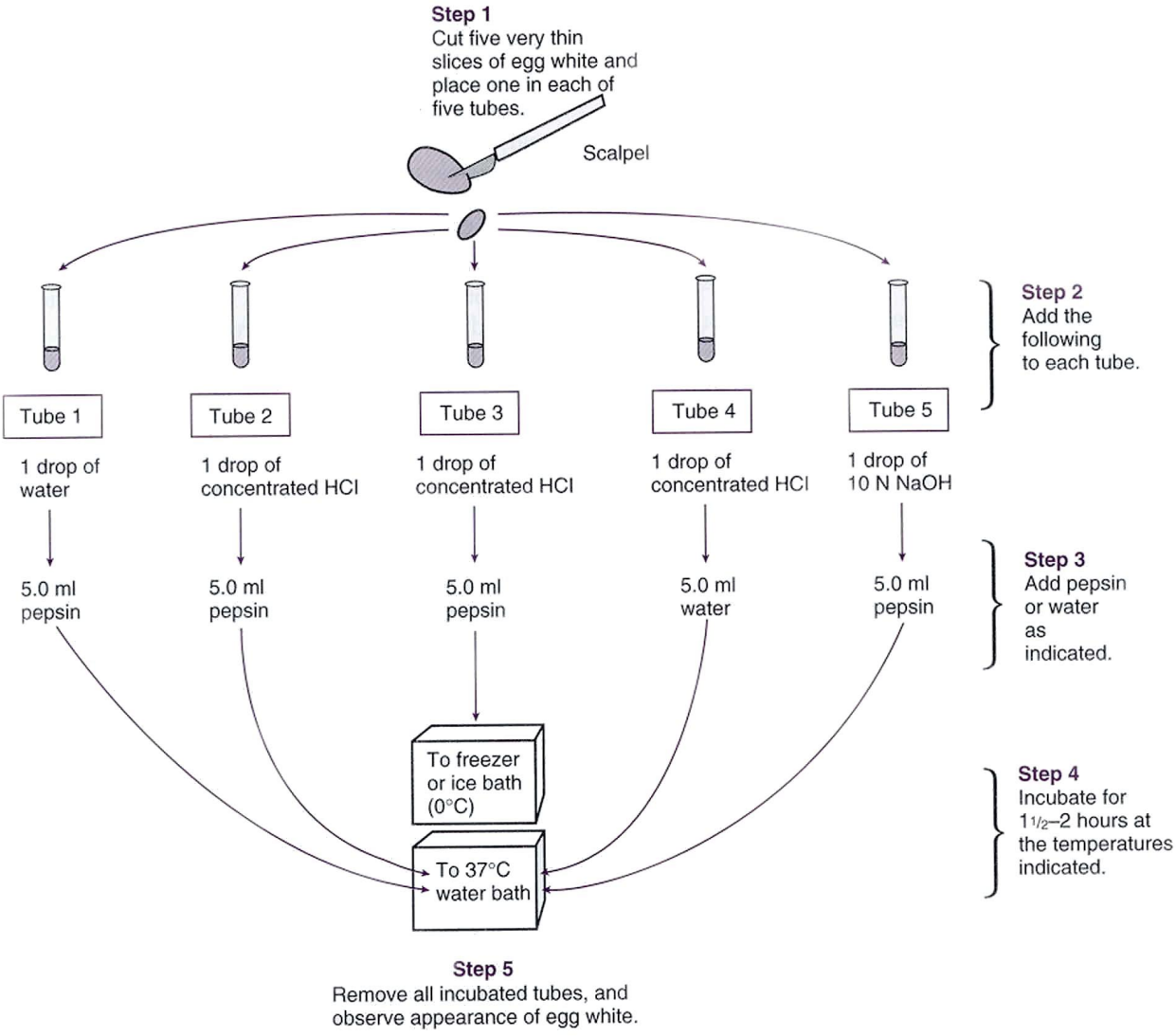
8. Divide the content of each sample by pouring half into four new test tubes
9. Test one set of four solutions for **starch** by adding a three drops of Lugol's reagent. The presence of starch is indicated by the appearance of purplish-black color.

10. Test the other four solutions for **maltose** as follows

Color	Amount of Maltose (Arbitrary Units)
Blue	none
Green	1
Yellow	2
Orange	3
Red	4

- Add 4 ml Benedict's reagent to each of the four tubes and immerse them in a boiling water bath for 5 minutes
- After the incubation, note the color of the solution and record the glucose content using the scale on the left

PART II: DIGESTION OF PROTEIN BY PEPSIN



1. Label 5 test tubes
2. Cut five slices of egg white as thin as possible; the slices should be about the same size
3. Place a slice of egg white in each tube

4. Add 1 drop distilled water to tube 1

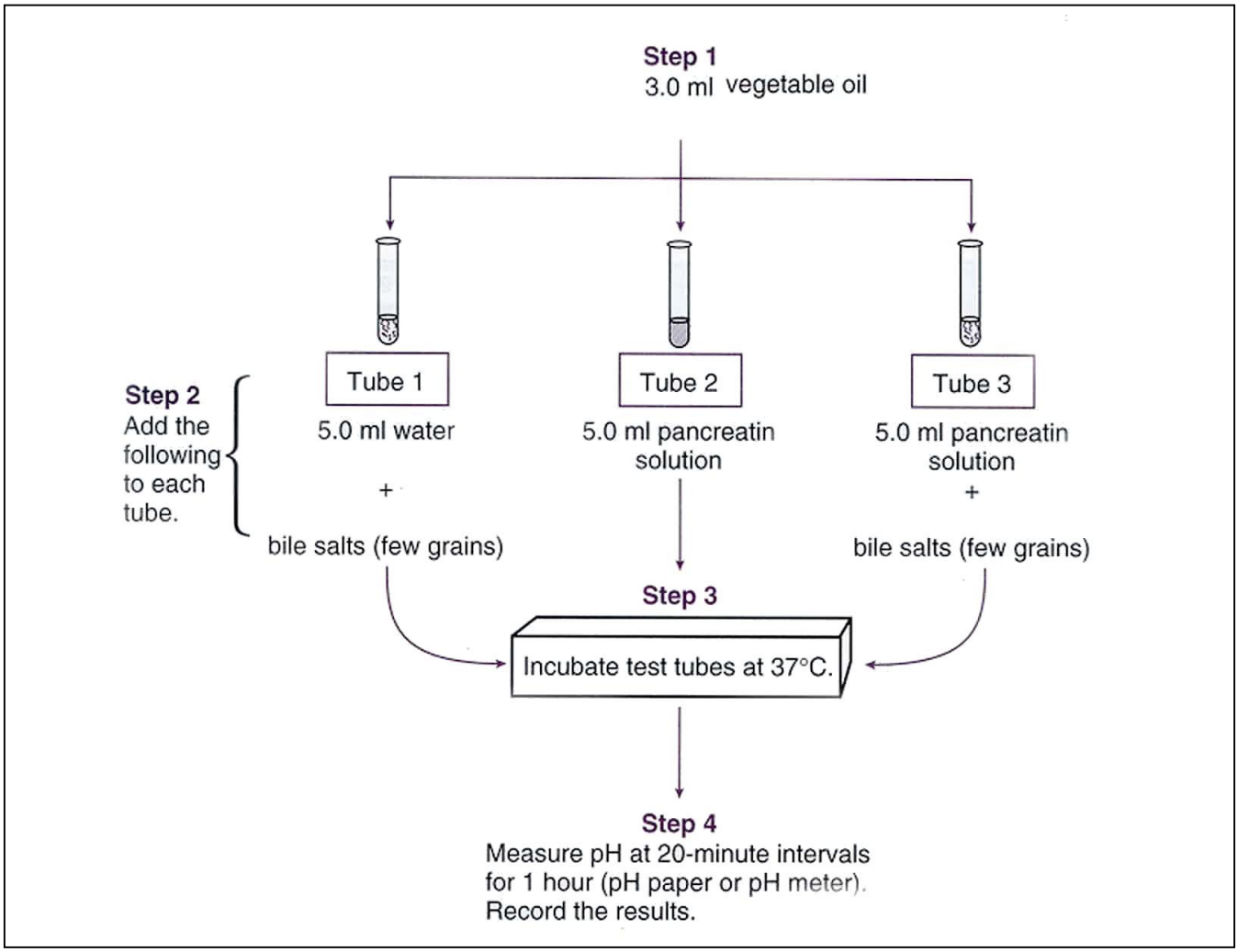
5. Add 1 drop HCl to tubes 2, 3, and 4
6. Add 1 drop of concentrated NaOH to tube 5

7. Add 5 ml pepsin solution to tubes 1,2,3, and 5
8. Add 5 ml distilled water to tube 4

9. Place tubes 1,2,4, and 5 in a 37^{o}C water bath. Place tube 3 in the freezer.
10. Incubate all tubes for 1 h.

11. Remove all tubes and record the appearance of the egg white on your data sheet

PART III: DIGESTION OF FAT BY PANCREATIC JUICE AND BILE



1. Add 3 ml vegetable oil to 3 test tubes

2. Add 5 ml water and a few grains of bile salts to tube 1
3. Add 5 ml pancreatin solution to tube 2
4. Add 5 ml pancreatin solution and a few grains of bile salts to tube 3

5. Incubate the tubes at 37^{o}C for 1 h checking the pH of the solutions at the beginning of incubation and every 20 min thereafter. Record your data

DATA SHEET. Lab 7. Digestion NAME:_____

PART 1: <u>The effects of pH and temperature on the activity of amylase</u>

	Starch	Maltose
Tube 1 water		
Tube 2 Amylase		
Tube 3 Amylase + HCl		
Tube 4 Boiled amylase		

1. Which tube contained the most starch and which the most maltose? What is your conclusion?

2. Reviewing your data, predict what would happen to salivary amylase activity was saliva was swallowed. Explain.

3. What is the effect of cooking on enzyme activity? Explain why this effect is produced.

PART 2: <u>Digestion of protein by pepsin</u>

	Appearance of egg white after incubation
Tube 1 Protein pepsin at 37 C	
Tube 2 Protein + pepsin + HCl at 37 C	
Tube 3 Protein + pepsin + HCl at 0 C	
Tube 4 Protein + HCl at 37 C	
Tube 5 Protein + pepsin + NaOH at 37 C	

1. Which test tube showed the most digestion? What do you conclude about the pH optimum of pepsin?

2. Using the results of this exercise, explain why food that is frozen keeps longer than food at room temperature.

3. Why stomach doesn't digest itself? Why doesn't gastric juice digest the duodenum?

PART 3: <u>Digestion of fat by pancreatic juice and bile</u>

	pH		
Time	Tube 1: Fat + Bile	Tube 2: Fat + Pancreatin	Tube 3: Fat + Bile + Pancreatin
0 min			
20 min			
40 min			
60 min			

1. Explain why digestion of fat should affect the pH of the solution.

2. Does bile digest fat?

3. In which tube did fat digestion occur most rapidly? Why?

4. A person has jaundice and an abnormally long clotting time. Explain the relationships between these observations.

Laboratory Exercise 8: Electromyography

The activation of motor units can be studied by **electromyography**, the process of recording the electrical activity of muscle on a cathode-ray oscilloscope, polygraph, or computer. This may be done in humans by using small metal discs on the skin overlaying the muscle as the pick-up electrodes (external recording) or by using hypodermic needle electrodes inserted directly into the muscle. We are going to use the former method. The record obtained with such electrodes is the **electromyogram** (EMG). With needle electrodes, it is usually possible to pick up the activity of single muscle fibers. EMG activity is usually low when the muscle is at rest and there is a gradual increase in EMG activity when the muscle is contracted. There are several pathologic conditions that can be revealed with the EMG especially by using hypodermic electrodes.

We have five work stations for EMG recordings. You will work in pairs. EMG recordings will be taken from one person per pair. See attached instructions for the description of the exercise.

PART I.

You will perform the **"L01-EMG-1"** lesson on your computer. Follow the instructions in the attached handout. When you are finished, click on "Suspend". Leave the EMG electrodes on and continue with Exercise 2.

PART II.

Have the subject measure the maximal grip force (kg) for both right and left hands using the **dynamometer**. Record the results on the data sheet. Calculate 75% of the greatest grip force.

Click on "Resume".

a. First, record EMG from the relaxed forearm (dominant hand).
b. Record EMG using dynamometer. Repeat Clench-Release-Wait cycles, holding the clench for 2 seconds and waiting 2 seconds after releasing before beginning the next cycle. Begin with 10 kg clench force and increase in 10 kg increments for each cycle until maximum clench force is reached. Click on "Suspend" at the end of recording. Take 30 second rest before going to the last part of the exercise.
c. Clench the hand dynamometer with your maximum force and try to maintain it. When the maximum clench force has decreased by more than 50%, stop recording.

Analyze your recording and enter your data your data worksheet.

DATA SHEET. Lab 8. EMG NAME:_____

Part 1. Please fill out the data report sheet attached to your EMG instruction handout.

Part 2.

Peak #	**Force** (kg)	**Raw EMG** [CH 3] p-p	**Int. EMG** [CH 40] mean
1	rest		
2	10		
3	20		
4	30		
5	40		
6	50		
7	60		
8	70		
9	80		
10	90		

Maximum Clench Force: right hand	
Maximum clench force: left hand	
Time to fatigue (CH 40 delta T)	

Laboratory Exercise 9: Electrooculography

Eye movements can be recorded as an electrooculogram, a recording of changes in voltage that occur with eye movements. See attached handout for details.

Please fill out the data report sheet attached to your EOG instruction handout

Laboratory Exercise 10: Electroencephalography

Electrodes placed on the scalp above various regions of the brain can detect the electrical activity associated with functioning neurons, predominantly the summated postsynaptic potentials of the cortical neurons (pyramid cells). Recording the brain's electrical activity by using electrodes is called ***electroencephalography***, the recorded trace is called electroencephalogram (EEG).

EEG does not show regular easy-to-analyze-and-measure pattern like ECG waves do. Rather, EEG waves that we see on the trace is a complex mixture of various wave forms. These wave forms can be grouped by their frequency. The slowest waves are the delta waves (0.5 - 4 Hz), a bit faster waves are the theta waves (4.5 – 7 Hz). Although some delta and theta waves are present in the EEG during wakefulness, they are typical during sleep. Faster waves are the alpha waves (7.5 – 13 Hz) and the fastest waves are the beta waves (13 – 30 Hz). Alpha waves dominate the EEG when we are awake and relaxed, beta waves are characteristic of high arousal, intense mental activity. When a relaxed person starts performing an intense mental task, his/her alpha waves will be replaced instantaneously by the fast beta waves, a phenomenon called ***alpha blockade***. The rule is that the higher the arousal level is, the faster the activity of the EEG and, vice versa, the deeper we sleep, the slower the EEG is.

Because EEG is a "chaotic mixture" of various waves, the individual wave forms that make up the EEG are practically indiscernible by simply looking at the recordings. Careful mathematical analysis of the EEG recordings, however, easily reveals the dominance (relative contribution) of various wave forms in the EEG. In the following exercise, you will record EEG and you will analyze the wave components of the recorded EEG.

PROCEDURES

Carefully **read you detailed handout** before staring the experiment. The following is only a brief summary of the major steps you will have to perform.

1. Place the EEG electrodes on your subject and connect them to the data collection box as explained on pages 8 - 9 in your handout. Pay close attention to "Hints for obtaining optimal data" and "Guidelines for electrode placement".

2. Ask your subject to completely relax with eyes closed for ~5 min before recording.

3. Start the BIOPAC Student lab program, choose Lesson 3 (L03-EEG-1)

4. Calibrate the hardware as explained on page 10.

5. Perform recordings (page 11 – 13). When you record, make sure that you **insert the markers** at the appropriate places. If you fail to do so, you won't be able to analyze your data later on.

6. Analyze your data (page 14 – 16)

7. Fill out your data report sheet

Laboratory Exercise 11: Polygraphy and Galvanic Skin Response

The human skin displays several forms of bioelectric phenomena, especially in areas of the extremities such as the fingers, palms of the hand and soles of the feet. When a weak electric current is steadily applied between two electrodes placed about an inch apart on the palm of the hand or on the fingers, the recorded electrical resistance between them (the ***galvanic skin resistance***) varies in accordance with the emotional state of the subject.

The physiologic basis of this change is a change in autonomic tone, largely sympathetic tone, occurring in the skin and subcutaneous tissue in response to a change in the affective state of the subject. Changes in autonomic tone alter sweating and cutaneous blood flow, which, in turn, change galvanic skin resistance. For example, increased sympathetic tone in response to a painful stimulus will elicit increased activity of the sweat glands. Increased sweating lowers the electrical resistance of the skin, because sweat contains water and electrolytes, both of which increase electrical conductivity of the skin. As in case of somatic stimuli (e.g., pain), changes in emotion elicit changes in peripheral autonomic tone and therefore in galvanic skin resistance. A common example is the vasodilation of cutaneous blood vessels of the face (blushing) and increased sweating that often occur in the emotional state of embarrassment.

The detection and recording of the galvanic skin resistance is often combined with the detection of other autonomic-dependent psychophysiological variables such as heart rate, respiratory rate and blood pressure. The device that detects and records these variables simultaneously is called a ***polygraph*** ("*poly*" = many, "*graph*" = write). You will perform polygraphic recording in this experiment, since you will record three physiologic variables simultaneously:

a. respiration
b. galvanic skin resistance
c. heart rate

The "lie detector" is a type of polygraph. Its principle is that changes in emotion that are associated with intentional falsification of answers to carefully selected and worded questions involuntarily alter autonomic output in such a way as to cause recognizable changes in recorded physiologic variables. The experiment you will perform, although the recording procedures and measures are similar to those used in "lie detector" tests, is not a lie a "lie detector test". All you will do here is to record the subject's physiological responses to certain questions.

PROCEDURES

Carefully **read you detailed handout** before staring the experiment. The following is only a brief summary of the major steps you will have to perform.

1. Place the respiratory transducer, ECG electrodes and the finger electrodes (galvanic skin response transducer) on your subject and connect them to the data collection box as explained on pages 7 – 10 in your handout.

2. Start the BIOPAC Student lab program, choose Lesson 9 (L09-Poly-1)

3. Calibrate the hardware as explained on pages 11 – 12.

4. You will record three segments of data, 120 min each. Before you begin recording please read the instruction for all three recording segment. Pay particularly close attention to "Hints for obtaining optimal data" on page 13. When you record, make sure that you **insert the markers** at the appropriate places. If you fail to do so, you won't be able to analyze your data later on.

5. Record your first segment of data as explained on pages 13 – 14.

6. Record the second segment (pages 15 – 16)

7. Record the third segment (pages 16 – 17)

8. Make a copy of your result on a floppy disk and analyze your data file on another computer. We have only two workstations for recording polygraphy; keep those two computers available for the actual recordings. Data can be analyzed on any of the other six computers.

9. Fill out your data report sheet.

Laboratory Exercise 12: Vision, Hearing, Tactile Senses

PART I. VISION

If you are wearing glasses perform these experiment while wearing them unless otherwise directed.

1. Near point: This is the minimum distance at which your eyes can focus on an object. Place the end of a ruler against your cheek, just below your left eye. Close your right eye. Place your finger against the scale and move it along the ruler until you find the minimum distance at which you can keep it focused. Record this as your near point for your left eye (in mm). Repeat this procedure with your right eye.

2. Visual acuity: This is how close two objects can be placed and still be seen as separate. This is measured by ***Snellen's chart***, which consists of letters of various sizes. The letters of certain sizes can be seen clearly at a specific distance by normal eyes. A person's visual acuity (V) is given by the equation:

   $V = d/D$

   where,
   d = the distance at which the tested person can read the letters
   D = the distance at which a normal eye can red the letters

   A normal eye is rated 20/20. This means that the letters that a normal eye can read from 20 feet the subject could also read it from 20 feet. Cover your right eye. Stand 20 ft. in front of the eye chart. Read the smallest line you can aloud to your lab partner. Have your partner record the letters you read and check your answers. The smallest line that you can read without making a mistake is your visual acuity for that eye. Repeat the procedure for the right eye.

3. Astigmatism: In an ideal eye, the refractive surfaces of the lens and the cornea would be spherical, with equal curvatures along all meridians. In many eyes, however, the corneal surface is not spherical, the curvatures are uneven, and there is a meridian of least curvature orthogonal to a meridian of greatest curvature. This distorts the path of the light rays entering the eyes, it causes an image to be sharp along one axis and blurred along another. Astigmatism can be revealed when a subject views a chart of radical lines.

   If you wear glasses, conduct the experiment without them. Stand 8 feet in front of the astigmatism chart. Cover your right eye and stare at the center of the chart. If all lines on the chart are equally clear and dark, then there is no astigmatism. If you perceive lines of one orientation to be in focus (e.g., the horizontal line), whereas lines at orthogonal orientations appear out of focus (e.g., vertical line), you have astigmatism. Repeat this procedure for the right eye.

4. Visual field: this is defined as the entire area in space seen by one eye when the eye is fixed in one position, i.e., everything that one can see without moving the eye. The instrument used to map the visual field is the ***perimeter***. Have the subject place his/her cheek against the

black plastic support so that the left eye is center in the mirror. Keep the right eye covered. The subject should stare only at the mirror with the left eye. Little disks of various colors serve as stimuli for determining visual field. Randomly choose one of the red, green, or white discs and place it behind the subject's head. Set the perimeter in horizontal position (0^0). Slowly move it along the perimeter towards the front of the eye. Have the subject tell you when he/she first sees the disc. Record this position in degrees on the special circular chart paper provided. Continue moving the disc toward the front and have the subject tell you when he/she first can tell the color of the disc. Record this second position on the chart, as well. Repeat the measurement using the same color disc but at different perimeter positions (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 degrees).

PART II. HEARING

The sensory organ for hearing is the ear. The VIII. cranial nerve transmits the sensory information from the ear to the brain. The ear has three major parts. The outer ear and the middle ear are essentially passive mechanical systems, they amplify the sound waves. A chain of three delicately suspended bones in the middle ear (the ossicles) are particularly important for amplification. The inner ear contains the cochlea. The cochlea houses the receptors the activation of which will generate action potential in the auditory nerve. Defects in the outer ear or the middle ear can cause conductive hearing loss. Most commonly, the tiny joints between the ossicles may become immobilized. Consequently, the amplification function of the middle ear is impaired. Defects in the receptors or the auditory nerve cause sensory-neural hearing loss. In this case, the mechanical, amplification function of the middle ear is intact, but the receptor-nerve component cannot translate the mechanical stimuli into neural activity. Simple hearing tests using a tuning fork can reveal if one has conductive or sensory-neuronal hearing loss. The principle of these tests is the following. Normally, sound waves reaches the outer ear through the air. This is called "***aerial conduction***". The middle ear amplifies the resonance and transmits it to the inner ear. There is a second mechanism, however, that also contributes to the stimulation of receptors. The cochlea is located inside the thick and dense wall of the temporal bone of the scull. If a stimulus sets the temporal bone into vibration then the vibration spreads directly onto the inner ear (bypassing the outer and middle ears) and causes vibration in the cochlea thereby activating the receptors. This mechanism is called "***bone conduction***". Aerial conduction is more sensitive than bone conduction. Bone conduction does not play an important role in every day life except under one condition: when you speak and hear your own voice. When you speak, your temporal bone is set into vibration by your own voice. What you perceive is a mixture of bone-conducted and air-conducted hearing. When you listen to your voice played from a tape recorder, it sounds strange and foreign because you hear it only through aerial conduction.

1. Weber's test: Strike the tuning fork with your palm and place the handle of it on the middle of the forehead of the subject. If hearing is normal, the subject should report that the sound is equally loud in both ears. Have the subject simulate conduction deafness by placing his/her index finger in his/her right ear. Repeat the test. Which ear has the loudest sound now?

2. Rinne's test: In this test the bone conduction and aerial conduction of the same ear are compared. Place the handle of the vibrating tuning fork on the mastoid process of the left temporal bone (behind the left ear). You will hear the fork vibrating. When you don't hear the vibration any longer then move the fork to within several inches from the ear. What

happens to the sound? What would you expect in sensory-neural hearing loss? What would you expect in conductive hearing loss?

PART III. TACTILE SENSES.

The tactile threshold is the minimum intensity of a stimulus that can be consciously detected. Cutaneous acuity is how close two points can be applied to the skin and still be felt as two distinct points. Conduct the tests listed below on both index fingers, palms, backs of both hands, both sides of the forehead and neck. The aim is to determine which area has the highest tactile sensitivity and the highest tactile acuity.

1. Relative touch sensitivity: Have the subject close his/her eyes. Apply bristle #1 of the aesthesiometer to the forehead with enough tension so that it bends only slightly. Ask the subject if he/she can feel the pressure. If not, apply bristle #2. Keep applying the bristles until you find one that the subject can feel. Record the number of that bristle.

2. Two point discrimination: Have the subject close his/her eyes again. Apply the caliper to the skin with the points 5 cm apart. Ask the subject to state if she/he feels one or two points. If the subject reports "two", close the caliper by 5 mm, and repeat the experiment. Continue this procedure until the subject can detect only one point. Record the distance between the caliper tips when this is obtained.

DATA SHEET. Lab 12. Sensory Physiology NAME:_____

Part I. Vision

Left eye near point: Right eye near point:

Left eye acuity: Right eye acuity:

Left eye astigmatism: Y / N Right eye astigmatism: Y / N

Part II. Hearing

Weber's test:

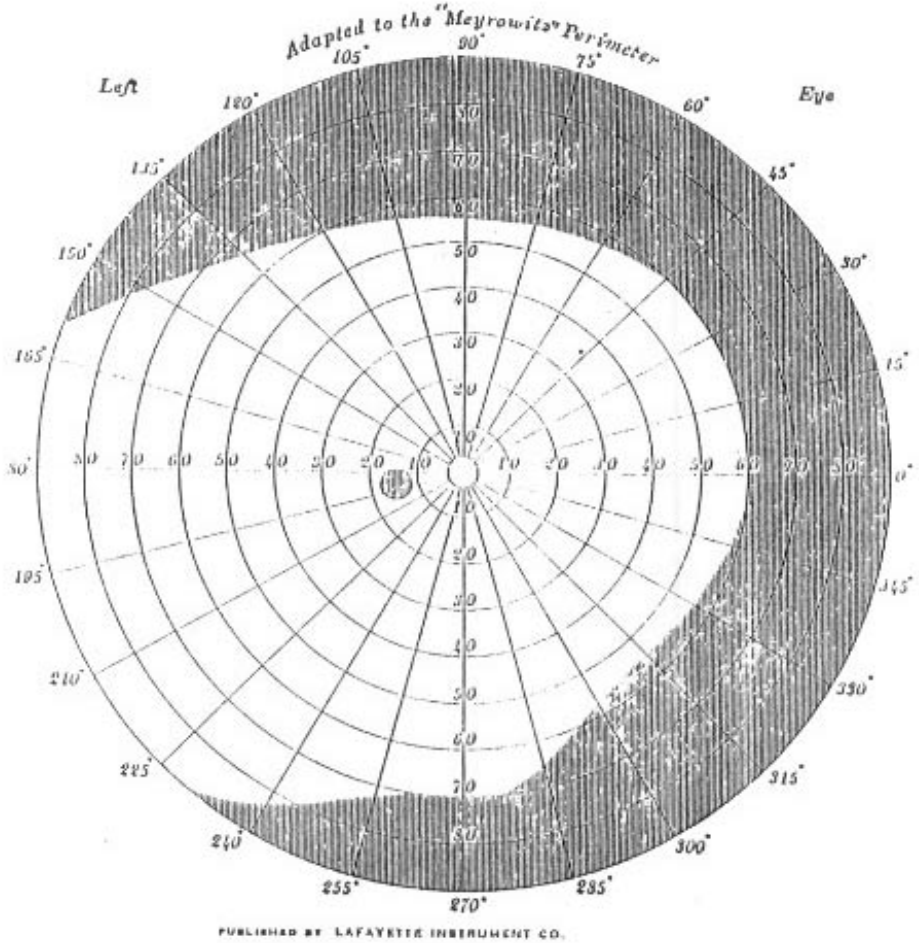
Rinne's test:

Part III. Tactile senses

	Bristle Number			Two-Point Threshold		
	Left Side	**Right Side**	**Mean**	**Left Side**	**Right Side**	**Mean**
Finger						
Palm						
Back of hand						
Forehead						
Neck						

Which area has the greatest sensitivity? Which has the least?

Do the two sides of the body differ in tactile sensitivity?


Adapted to the "Meyrowitz" Perimeter
Left
Eye
90°
75°
60°
45°
30°
15°
0°
345°
330°
315°
300°
285°
270°
255°
240°
225°
210°
195°
180°
165°
150°
135°
120°
105°
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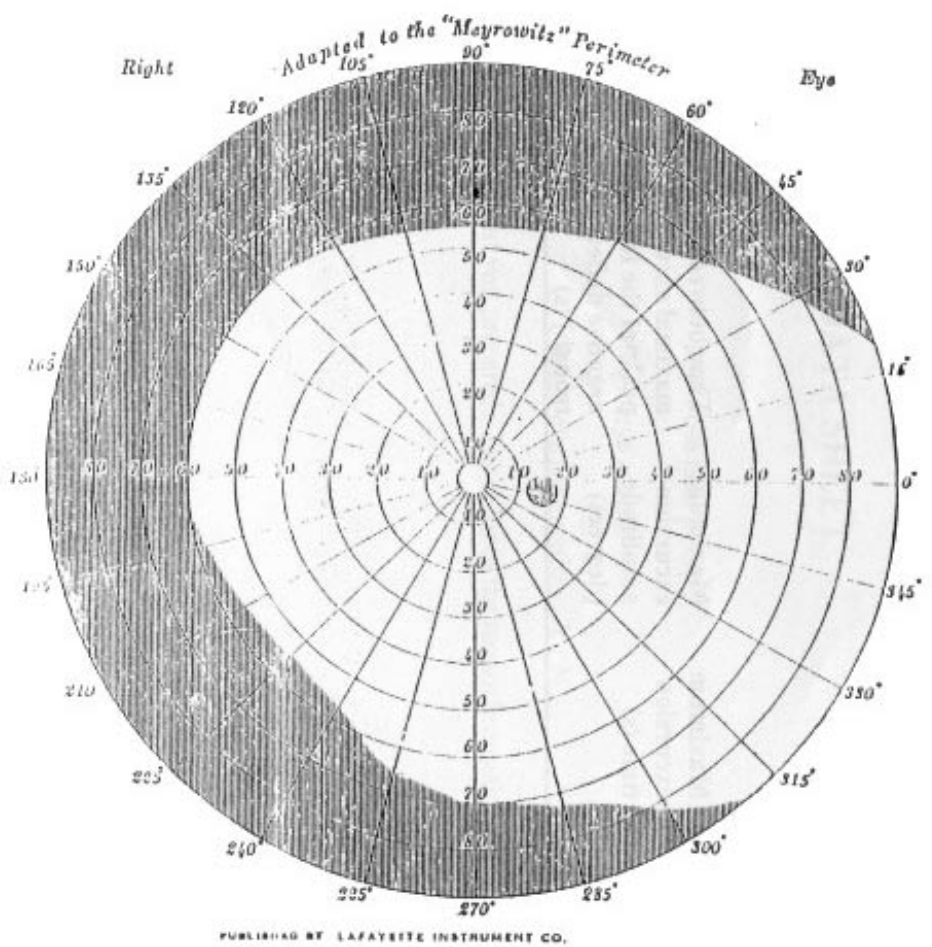
PUBLISHED BY LAFAYETTE INSTRUMENT CO.


Adapted to the "Meyrowitz" Perimeter
Right
Eye
90°
75°
60°
45°
30°
15°
0°
345°
330°
315°
300°
285°
270°
255°
240°
225°
210°
195°
180°
165°
150°
135°
120°
105°
50
60
70
80
50
40
30
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70
80
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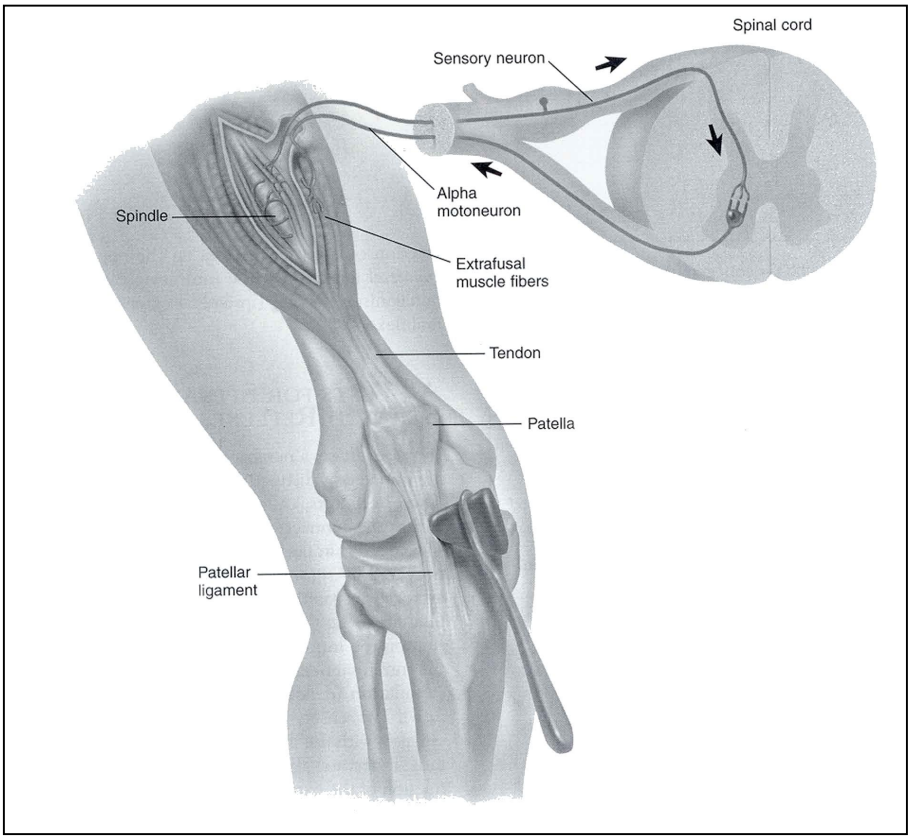
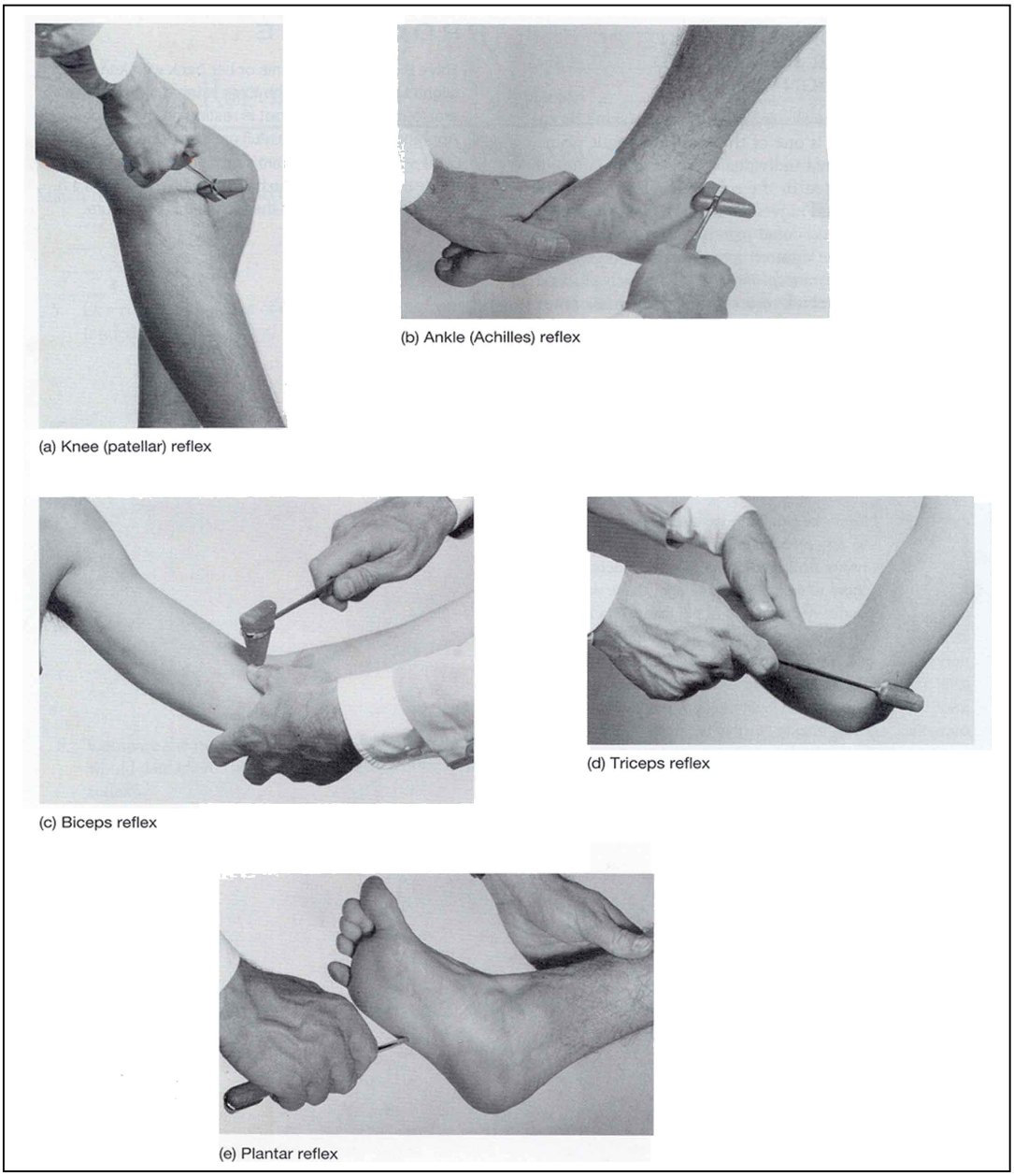
Laboratory Exercise 13: Stretch Reflexes

When a skeletal muscle with an intact nerve supply is stretched, it contracts. The response is called the **stretch reflex**. The stimulus that initiates the reflex is stretch of the muscle, and the response is contraction of the muscle being stretched. The receptors are the muscle spindles which are interspersed among the working muscle fibers. The impulses from the spindle are conducted to the central nervous system by fast sensory fibers that pass directly to the motor neurons which supply the same muscle. Stretch reflexes can also be elicited from most of the large muscles of the body. Tapping on the tendon of the triceps brachii causes an extensor response at the elbow as a result of reflex contraction of the triceps; tapping on the; and a tapping on the side of the face causes a stretch reflex in the masseter.



1. <u>Patellar (knee jerk) reflex</u>. Tapping the patellar tendon elicits the **knee jerk** reflex, a stretch reflex of the quadriceps femoris muscle.

You will measure the intensity of patellar reflex before and after exercise. There are four workstations available, you need to share them. Calibrate the sensors and fix them around the knee as will be shown in the lab. Have the subject sit on the lab bench with both legs hanging freely. Lightly (!) tap the subject with the percussion hammer just below the patella (kneecap). Using the computer, record three responses. Save the results on a floppy disk. Take the sensors off the subject and have him/her exercise by climbing the stairs from the basement to the third floor. Repeat the above test, save your second set of data. Perform the analysis.



2. **<u>Achilles (ankle jerk) reflex</u>**. Tapping the Achilles tendon elicits the reflex contraction of the gastrocnemius muscle. Have the subject kneel on a chair with his/her back to you, and with feet (shoes and socks of) projecting over the edge. Strike the Achilles tendon at the level of the ankle and observe the resulting plantar extension of the foot.

3. **<u>Biceps reflex</u>**. With the subject's arm relaxed but fully extended on the desk, gently press on his/her biceps tendon in the antecubital fossa with your thumb and strike *your finger* with the mallet.

4. **<u>Triceps reflex</u>**. Have the subject lie on her/his back with the elbow bent, so that the arm lies loosely across the abdomen. Strike the triceps tendon about 2 inches above the elbow.

DATA SHEET. Lab 13. Stretch reflexes NAME:_____

1. Before exercise

	Latent period (s)	Maximum excursion time (s)	Maximum excursion amplitude (degrees)	Time between the peak of maximal excursion and the peak of the following negative deflection (i.e., first trough) (s)	The amplitude of the first negative deflection (degrees)
Test 1					
Test 2					
Test 3					
Average of 3 tests					

2. After exercise

	Latent period (s)	Maximum excursion time (s)	Maximum excursion amplitude (degrees)	Time between the peak of maximal excursion and the peak of the following negative deflection (i.e., first trough) (s)	The amplitude of the first negative deflection (degrees)
Test 1					
Test 2					
Test 3					
Average of 3 tests					
Differences between post- and pre-exercise averages					

Laboratory Exercise 14: Rat Dissection

Warning:

The preservative solution is toxic. Avoid direct contact with skin or eyes. Do not lean close to the animal while dissecting. Wear gloves all the time.

Absolutely no eating and drinking in the lab during dissection exercises please!

You will work in pairs. You perform the dissection of the organs in the thoracic cavity, abdominal cavity and the organs of the reproductive system.

Thoracic cavity: Read the introduction in your dissection manual (page 1-3). Make the appropriate skin incisions (Figure 1.3) but do not perform the dissection of head-neck-upper limb-lower limb muscles (skip page 4-15). Instead, go to "Exercise 2" and follow the instructions in your manual for "Exercise 2" (page 17), "Exercise 4" (page 25), and the dissection of thyroid glands, parathyroid glands and thymus ("Exercise 6", page 35).

Abdominal cavity: Organs of the digestive, urinary and reproductive systems, endocrine organs and blood vessels in the abdominal cavity. Follow the instructions in your dissection manual (Exercise 3, 5, and 6).

When you finished, label your animal and place it back to the preservative solution. We will use the same animals for lab exam.

On the lab exam, you will be tested on your ability to identify various anatomical structures on the dissected animals you and your classmates prepared. You will be asked to identify the following structures ("parts" means that you will have to identify various parts of that organ):

Vessels	Organs in the neck and thoracic cavity	Organs in the abdominal cavity	Reproductive systems
v. cava superiors v. cava inferior pulmonary trunk coronary vessels ascending aorta aortic arch brachiocephalic artery carotid arteries subclavian arteries brachial arteries thoracic aorta abdominal aorta celiac trunk renal arteries and veins superior and inferior mesenteric arteries gonadal arteries and veins common iliac arteries and veins femoral arteries and veins portal vein	larynx trachea thyroid gland esophagus heart (parts) bronchi lungs (parts) thymus diaphragm	stomach (parts) pyloric sphincter duodenum ileum jejunum colon (parts) cecum rectum anus mesentery omentum major pancreas liver (parts) spleen adrenals kidney (parts) ureter urinary bladder urethra	vagina uterus (parts) uterine tubes ovaries seminal vesicle prostate epididymis testes ductus deferens scrotum penis

Laboratory Exercise 15: Journal Club

Objective: The "Journal Club" has two objectives. First, you experience how to prepare and give a short, scientific presentation. Second, you will experience how to work in a scientific team the members of which are not chosen by you but assigned by someone else.

The class is divided into teams of 3-4 students. Each team receives one research paper from various topics in physiology. The teams will present these papers on November 24, at our mini science meeting. About 10-20 min is allocated for each presentation. You are encouraged to prepare PowerPoint presentations, transparencies, handouts, anything that may help your audience follow and understand the presentation. Each team member has to be fully prepared because one of the team members (and only one) will be chosen randomly to give the presentation. After the presentation, the entire class will discuss the paper. The audience will ask questions related to the presentations and a member of the presenting team (anyone, not necessarily the one who gave the presentation) will answer.

Grading: The emphasis is on team work. Each member of a team is equally responsible for preparing the presentation, answering the questions and participating in the discussion of other papers. Therefore, everyone from the same team will receive the same grade (0-30 points). This exercise counts as the third lab exam (13.64% of your final lab grade). The following factors will be equally weighed in your grade:

1. The content of the presentation. What information is conveyed and what supplemental material is prepared (e.g., figures, charts, etc.). If the presentation is significantly shorter than 10 min or longer than 20 min points will be taken. There is no time limit set on the following discussion.

2. How adequately the team answers questions.

3. How actively the team members participate in the discussion of other presentations.

What is **not** a factor in your grade:

1. You won't be graded as a performer. The important is what you say, not how you said it.

2. You are not expected to be able to answer all the possible questions during the discussion. You have to make a reasonable effort to deal with the questions, come up with an answer based on the information in the paper and your general knowledge of physiology.

Material: All research papers will be uploaded to ERes.

Help: First of all, team members should work together, help each other understand the papers and prepare for the presentation. It is possible that you will have difficulties understanding part of your paper, the techniques used in the research, the significance of the study, the pig picture where the study fits in, etc. I will be available for help if you contact me.