ZOOL 142L

1

Endocrine System and Stress Response

Lab Manual Exercises: Exercise 27 (Activities 1& 2)

Learning Outcomes:

1. Identify major locations of major endocrine organs using human torso model.
2. Identify histological sections of selected endocrine organs.
3. List hormones produced by each endocrine organ and describe their effects on the human body.
4. Review the functional stages of stress response
5. Compare the effects of different stressors on activity of the autonomic nervous system.

Materials:

	Endocrine Slides (see below):		Microscope Accessory Boxes (10)
	Ovary		Stopwatches
	Pituitary		Step Boxes
	Adrenal Gland		Pain Stimulator (Cheese Grater w/dowel rods)
	Thyroid		Digital Metronomes (2)
	Hypothalamus		Stereo Speakers or Headphones (2)
	Testis		Ipod w/ distracting music (2)
	Automatic Blood Pressure Cuffs (5)		Math Problems (10 sets)
	Dissectible Human Torso		Excel Datasheet

Suggested Reading: Exercise 27; Lectures 1-2; Marieb & Hoehn Ch. 14

Assigned Tasks: (Lab Manual)

Activity 1: Identifying Endocrine Organs (p 408)

Identify the indicated endocrine organs on the human torso model and be familiar with the hormones they produce (no write-up required)

Pituitary* Thyroid*

Pancreas* Ovary* Testis*

Activity 2: Microscopic anatomy of Endocrine Glands (p. 409)

Examine histology of glands indicated with "*". For each:

1. Be able to recognize each organ from histological sections.
2. Identify major endocrine structures or cells (e.g., eyelets of langerhans)
3. Know hormones produced by tissue and their effects

Part B: Role of A.N.S. and Endocrine System in Stress Response

Purpose: To understand the physiological basis of the Stress Response

Directions:

1. Form lab groups of 4-5 students
2. Choose **TWO** of the following stressors (Labeled A-E)
3. Research Question: What effect will the stressor have on heart rate (HR) and blood pressure (BP)?
4. Form a hypothesis for each stressor tested (e.g.," Pain will elevate HR")
5. Make measurements as directed and record on spreadsheet (included)
6. Construct a bar graph depicting HR & BP before, during, and after the stress in applied (be sure to **average** group data- don't draw a bar for each person)
7. **Write a formal lab report describing your experiment** (refer to report format). You conclusion should include a statement as to which hormones (if any) or division(s) of the ANS are responsible for the observed change in vital signs.

Stressors (Choose 2)

A. Temperature Stress

1. Have the subject sit quietly for 5 minutes.
2. Record resting HR and BP. Deflate cuff but leave attached to one arm.
3. Ask the subject to place their hand in a bucket of ice water.
4. Take HR and BP measurements at 1 minute intervals for 3 minutes.
5. At 3 minutes, have subject remove their hand from the ice water and pat dry.
6. Record post-stress HR and BP 2 min after removal.

B. Orthostatic Hypotension

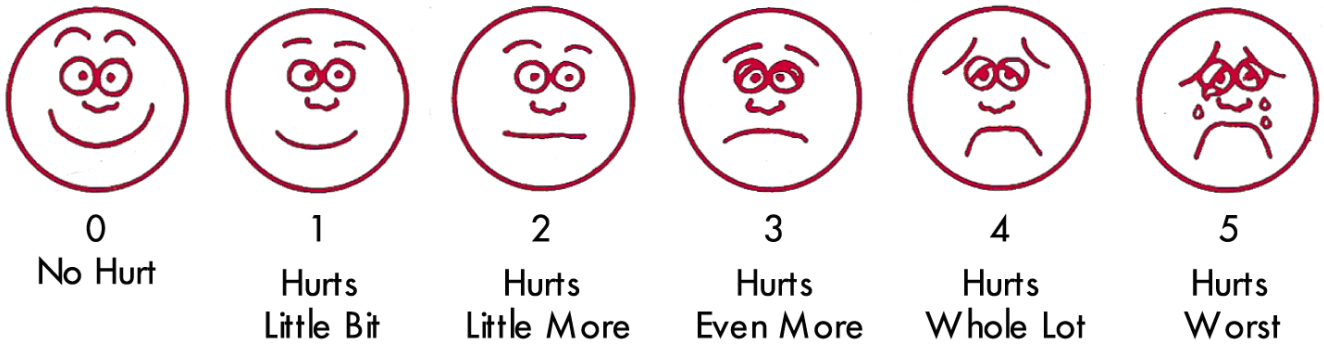
1. Have the subject sit quietly for 5 minutes.
2. Record resting HR and BP. Deflate cuff but leave attached to one arm.
3. Ask the subject stand upright against the wall. It is important that they relax as much as possible (other group members should remain quiet!).
4. Record HR and BP immediately upon standing.

SAFETY NOTE: It is important that other group members closely monitor the study subject and be prepared to catch them should they faint.

5. Measure HR and BP at 2 min intervals (maximum of 8 minutes). Stop the experiment if the subject begins sweating or feels light-headed.
6. At the end of the time period, have the subject sit down and rest. Record post-stress HR and BP 2 min later.

C. Pain

1. Have the subject sit quietly for 5 minutes.
2. Record resting HR and BP. Deflate cuff but leave attached to one arm.
3. Place the plastic plate on the subjects shin with the wooden dowels facing inward.
4. Attach the supplied pressure cuff over the plate and inflate gradually until the subject reaches a pain intensity of 3 on the Wong-Baker Pain Rating Scale (below). Note the pressure on the cuff and maintain this throughout the study (do not exceed 110 mm Hg).
5. Record HR and BP at 1 minute intervals for a maximum of 3 minutes.
6. Remove assembly at 3 minutes or when subject requests.
7. Record post-stress HR and BP 2 min after removal.



C. Exercise Stress

1. Have the subject sit quietly for 5 minutes.
2. Record resting HR and BP. Deflate cuff but leave attached to one arm.
3. Set metronome at 98 beats/ minute. Have subject step up and down on the step-box, keeping pace with the metronome (approximately 24 steps/ min).

4. Record HR and BP at 1 minute intervals for 3 minutes. It will be necessary for a student to support the sphygmomanometer during the exercise. The experiment should be terminated if the student feels faint or out of breath.
5. Stop exercise at three minutes and have the subject sit and rest.
6. Record post-stress HR and BP 2 minutes after end of exercise.

E. Noise Stress

1. Have the subject sit quietly for 5 minutes.
2. Record resting HR and BP. Deflate cuff but leave attached to one arm.
3. Ask subject to complete sheet of math problems as quickly as possible (maximum time allowed = 2 minutes).
4. Record HR and BP. Grade math problems and record the number of correct answers.
5. Repeat steps 3-4 while listening to blaring, obnoxious music.
6. Record post-stress HR and BP 2 minutes after end of treatment.

	Pre-Stress Measurments		**Stress 1**		**Stress 2**		**POST-Stress Measurements**	
	(Baseline)		Math Only		Math + Loud Music		2-min post stress	
ID	BP	HR	BP	HR	BP	HR	BP	HR

		Pre-Stress Measurments		Stress Measurements								POST-Stress Measurements	
		(Baseline)		Time 1		Time 2		Time 3		Time 4		2-min post stress	
ID	**Stress Treatment**	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR	BP	HR

B – PROBLEM SET

POST-STRESS

MULTIPLICATION:

1. 36	2. 43	3. 57	4. 84	5. 73	6 67
x16	x21	x13	x17	x27	x37

7. 23	8. 71	9. 59	10. 112	11. 83	12. 127
x95	x33	x45	x59	x39	x63

DIVISION:

1. 588/21 =	2. 5888/92 =	3. 7956/102 =	4. 1800/75 =
5. 4816/56 =	6. 1536/32 =	7. 2852/31 =	8. 2178/66 =
9. 2783/23 =	10. 4686/33 =	11. 4387/107 =	12. 4088/56 =

A – PROBLEM SET

PRE-STRESS

MULTIPLICATION:

1. 25 x15	2. 38 x21	3. 42 x18	4. 28 x18	5. 32 x24	6 48 x32
7. 54 x36	8. 68 x44	9. 75 x24	10. 86 x56	11. 92 x64	12. 102 x78

DIVISION:

1. 384/12 =	2. 428/107=	3. 540/135=	4. 375/15=
5. 576/18=	6. 375/25=	7. 1716/22=	8. 2108/34=
9. 1872/36=	10. 1911/21=	11. 3975/53=	12. 5695/67=

ZOOL 142L

2

Blood

Lab Manual Exercises: Exercise 29A (Activities 2,3,4,7,8,9)

Learning Outcomes:

1. Name the major components of blood.
2. Identify red blood cells and leukocytes using size- and stating properties and list the function(s) of each.
3. Measure hematocrit and discuss its physiological significance.
4. Perform ABO and Rh blood typing.
5. Discuss compatibility of blood types and consequences of transfusing mismatched blood.
6. Describe the composition and function of blood plasma and discuss its use in evaluating overall health of organ systems.

Required Materials:

	Biohazard Bags (4)		Safety Glasses (20)
	Phlebotomy Containers (4)		Synthetic Blood for Hematocrit
	Prepared Blood Slides (normal and pathological)		Synthetic Blood Typing Kits (4)
	Microhematocrit Centrifuges (2)		Toothpicks (1 box)
	Microhematocrit tubes (10 containers)		Lab Disinfectant
	Critoseal (10)		Paper Covers for Lab Benches
	Microscope Accessory Boxes (10)		Excel Datasheet
	PCV/HCT Reference Scale		Disposable Gloves
	Hand Tally Counters/ Differential Counter		

Optional Materials:

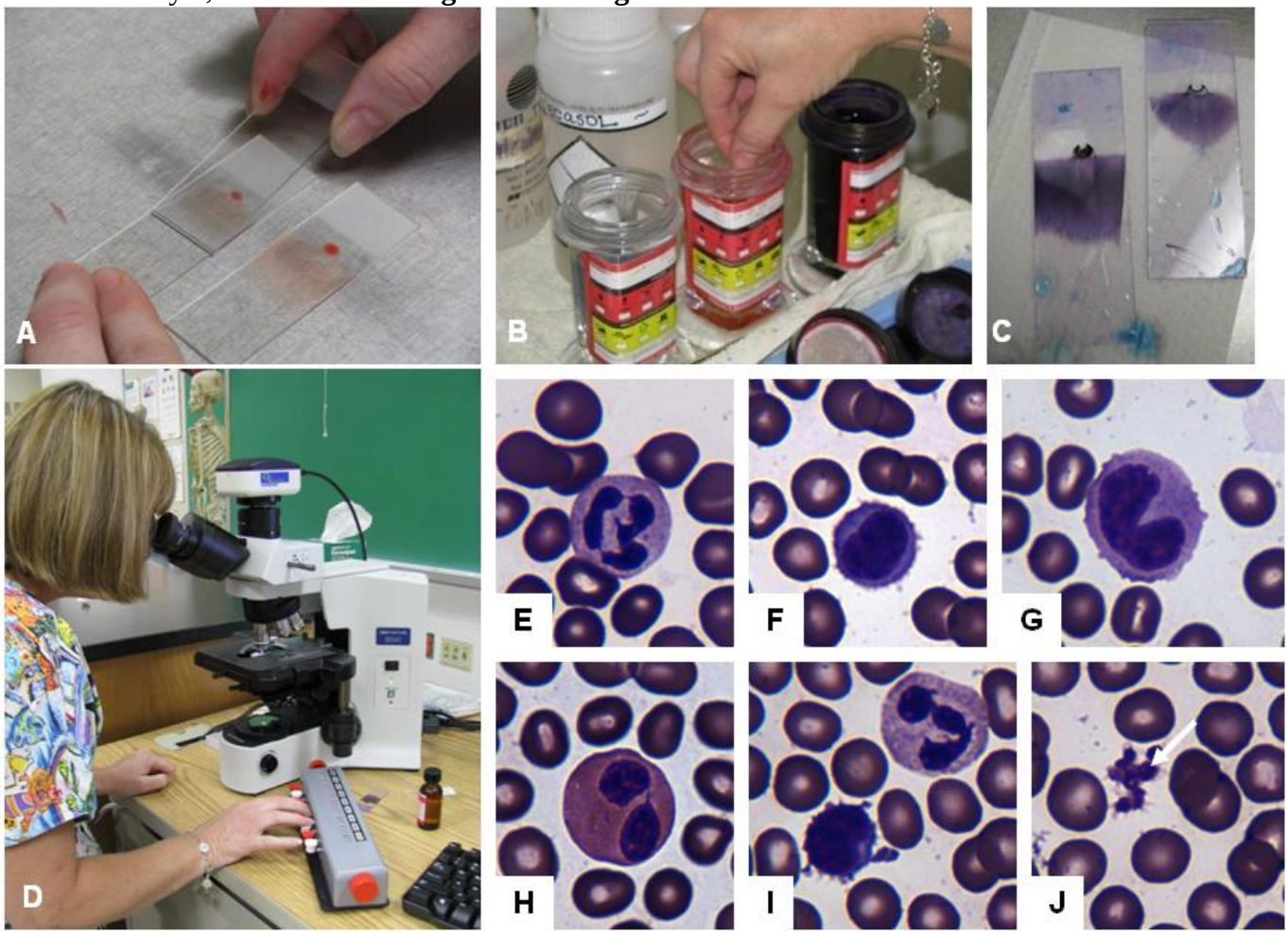
	Whole Animal Blood from a Vet Hospital (5)		Distilled Water Squirt Bottles (6)
	Refractometers (10)		250 ml beakers (6)
	Dip-Quick Stains 1-3 (2 sets)		Laser Cyte and Blood Chemistry Machines
	General Blood Chemistry Panels (2 sets)		LTT, RTT and green serum separators (4 ea)

Suggested Reading: Exercise 29A; Lectures 3-4; Marieb & Hoehn Ch. 17

Activity 2: Examining Formed Elements (p 426)

Obtain a human or animal blood smear and observe under the microscope.

1) Identify and draw (or photograph) at least 5 types of blood components. Use 400X or 100X TM.
2) Measure the diameter of each cell using the ocular micrometer. For each drawing, list TM and include scale bar. Record in Table 1.
3) Next to your drawing you should list the major **function**s of the formed element. If it is a leukocyte, state whether it is **granular or agranular**.



Activity 3.1. Procedures for making a blood smear and differential WBC count. (E- Neutrophil, F- Lymphocyte, G- Monocyte, H- Eosinophil I- Basophil J- Platelet)

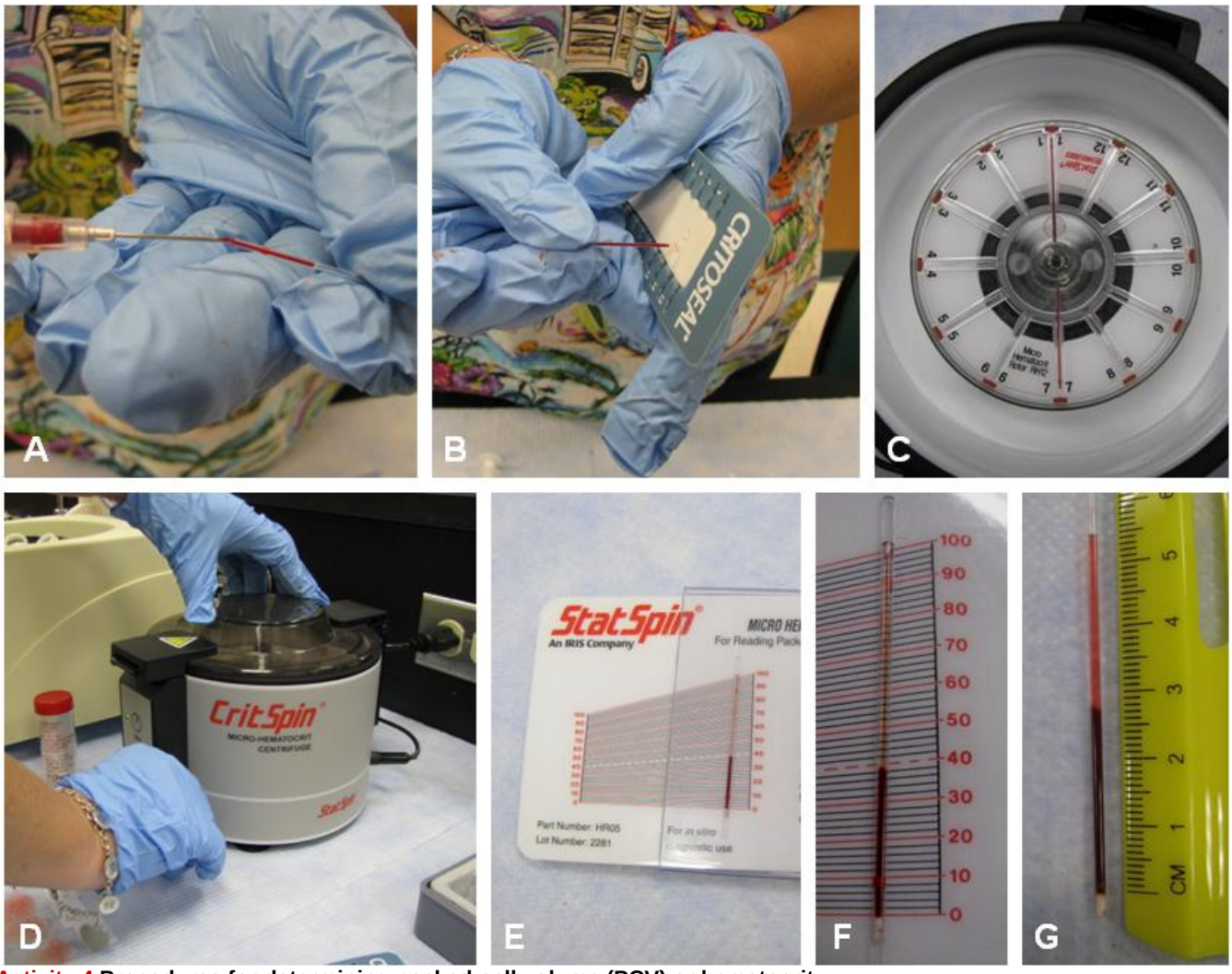
Activity 3: Conducting a differential WBC count (p 429)

The next step will be to determine the proportion of leukocytes in a blood sample and compare to normal ratios. This activity can be conducted with fresh or preserved blood smears. If using preserves smears, skip to "D".

A) Place a drop of fresh blood on one end of a microscope slide. Use another slide, placed at a 45 ° angle, to smear the blood across the first slide. Let dry 5 minutes. (Wear gloves!).
B) Dip for 1 minute each in Dip Quick stains 1-3.

C) Rinse with distilled water and let dry 5 minutes.
D) Examine the sample at 400 x under the compound microscope. Perform a differential white cell count following the instructions in your lab manual. Record number and % of each and record in table 2.
K) (Optional): Conduct a second differential count on the blood sample using the LaserCyte automated blood counter following the instructions of your professor.
L) Construct a graph comparing the proportion of each of the five leukocytes between the manual and automated counts (Figure 3.2).
M) **Conclusions:** Write a brief paragraph discussing your results. Did the manual count match the automated count? Are the blood counts within normal levels? If not, which blood cells were above or below normal? What type(s) of conditions or diseases do these suggest?

Figure 3.2 Performing a complete blood count using the Idexx® LaserCyte (A). Check that the green LED on the front of the LaserCyte is illuminated and ensure that adequate sheathing fluid is present. B) Collect blood sample into one of the IDEXX LTT's and load into slot #1 (C). Place a fresh GTT into slot #2. D) Enter patient data into the VetLab Station and initiate test by selecting LaserCyte icon. E) Once test is completed remove LTT and GTT and discard the latter as biohazard waste. Any sample remaining in the LTT is unaffected and can be used for additional blood counts or smears.



Activity 4 Procedures for determining packed-cell volume (PCV) or hematocrit.

Activity 4: Measuring the Hematocrit (p 430)

In the next activity, we will be measuring the hematocrit of fresh or synthetic blood.

A) Collect a small quantity of blood into a microhematocrit tube.
B) Plug one end with Critoseal.
C) Place into the microhematocrit centrifuge. Be sure to balance the rotor evenly by placing samples opposite one another.
D) Spin for the appropriate amount of time.
E-F) Remove sample and determine the PCV reading by comparing to the microhematocrit scale.
G) If sample is too small to use the microhematocrit scale, use a ruler to measure the distance from the bottom of the sample to the top of the packed cells (approximately 28 mm in the photo above). Divide this measurement into the length of the total sample (55 mm) to determine the hematocrit (28/55= .51 or 51%)
H) Record class results in table 3 and include in your lab report.
I) Construct a graph comparing the PCV values for all samples in the class.

J) **Conclusions:** Write a brief paragraph discussing your results. Did your sample fall within normal or abnormal levels? Is the PCV you recorded more typical of males or females? Did anyone have unusually high or low hematocrit readings? If so, discuss possible causes and health implications for the abnormal readings.

Activity 7: Typing for ABO and RH Blood Groups

In the next activity, we will be determining blood type using synthetic human blood.

1) Perform ABO and Rh blood typing using synthetic blood provided by your instructor. Follow the directions accompanying the blood typing kit. Photograph your blood card.
2) Pool class data on blood type and include in table 4.

4) **Conclusions:** Write a brief paragraph discussing your results. Given your blood type, which types of donor blood can you receive? Which recipients can receive your blood? Which blood types were most abundant in the class? How do these compare to the distribution of blood types in Hawaii?

Activity 10: Blood Chemistry Panels (Presenting group conducts)

1) Select one sample of fresh blood from the class for blood chemistry analysis.
2) Follow the directions below for preparing and analyzing the sample.
3) Record the results in table 5.
4) **Conclusion:** Compare the values for plasma components to normal levels. What organs are responsible for producing or regulating each component? Are the components in your sample within clinical norms? If not, what types of condition(s) do they suggest? (To answer these questions, you will need to do some library and internet work).

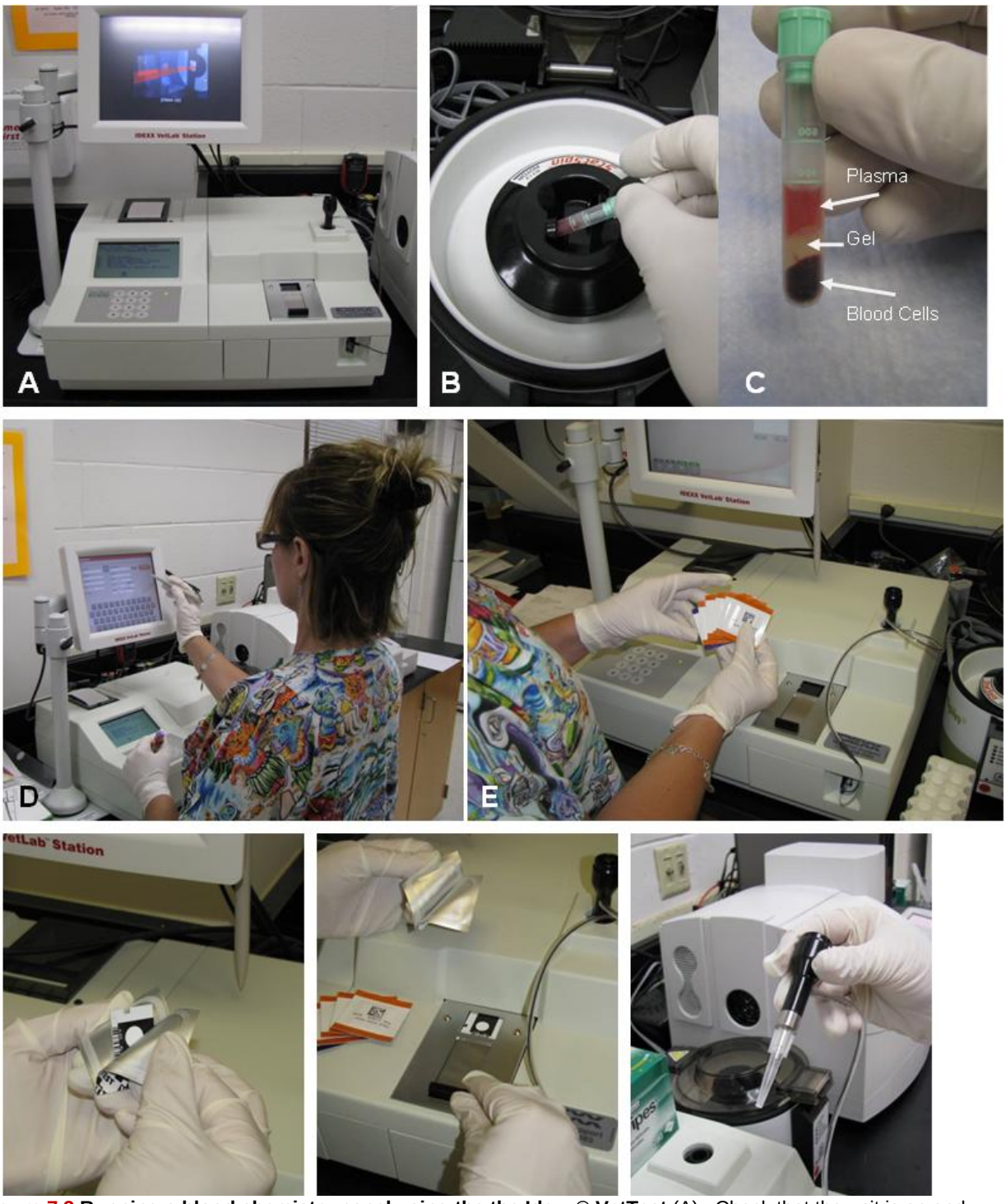


Figure 7.3 Running a blood chemistry panel using the the Idexx® VetTest (A). Check that the unit is on and that the cables for the pipetter are fastened securely to unit (arrow). B) If you have not already done so, aspirate 600 ul of whole blood into a plasma separator and centrifuge. Be sure to use a counterweight if necessary C) After centrificuation, the plasma will be at the top of the tube (red coloration of the pictured sample indicates presence of hemolyzed blood). D) Enter patient data into the VetLab station. It should load automatically into the VetTest. E) Remove the appropriate slides from freezer, peel open (F) and load each into the VetTest when prompted (G) Note that the notch (small arrow) in each slide faces left. H) When prompted, load a fresh pipette tip on to the pipetter and place gently into plasma sample. At one beep. Remove the pipetter from the sample after the double-beep sounds and replace into unit.

Table 1: Descriptions and functions of formed elements (From Activity 2):

Formed Element	Photo	Size and Staining	Functions
Erythrocyte			
Neutrophil			
Lymphocyte			
Monocyte			
Eosinophil			
Basophil			
Platelet			

Table 2: Comparison of manual and automated leukocyte counts (from Activity 3):

Leukocyte	Manual Count	Manual %	Automated Count	Automated %	Normal Values
Neutrophil					
Lymphocyte					
Monocyte					
Eosinophil					
Basophil					

Table 3: Comparison of hematocrit values among class samples (from Activity 4).

Sample	Normal or abnormal?
1	
2	
3	
4	
5	
6	
7	

Table 4: Comparison of blood types among class samples (from Activity 7).

Sample	Blood Type	Antibodies Present	Compatible Donors
1			
2			
3			
4			
5			

Table 5: Values of selected plasma components in a canine blood sample.

Component	**Source** (Where is it manufactured? Excreted?)	**Function**	**Normal Values**	**What would high values indicate?**	**What would low values indicate?**

ZOOL 142L

3

Anatomy of the Heart and Blood Vessels

Lab Manual Exercises: Exercise 30 (Activities 1-4) and 32 (Activities 1,3 & 4)

Learning Outcomes:

1) Identify the major anatomical structures of the heart and list their functions
2) Trace the pathway of blood through the pulmonary and systemic circuits
3) Identify and explain the functions of heart valves.
4) Identify histological sections of cardiac muscle.
5) Identify numerous tissue layers of arteries and veins and describe the functions of each.
6) Identify major arteries and veins of the human body.

Materials:

	Heart Models		Dissectible Human Torso
	Heart Posters		Preserved Sheep Hearts (4-5)
	Anatomy and Physiology Revealed CDs (10)		Dissection Instruments
	Cardiac Muscle Slides (10)		Compound Microscopes
	Container for tissue disposal		Laboratory Disinfectant
	Blood Vessel Slides (10)		Microscope Boxes
	Digital Projector		

Suggested Reading: Exercises 30 & 32; Lecture 5; Marieb & Hoehn Ch. 18

Assigned Tasks: (Lab Manual)

A. Cardiac Structure (Activity 1, p 447; Activity 2, p. 448): Using text book illustrations, organ models and "Anatomy and Physiology Revealed" CD, locate and identify required anatomy (see attached list). Make a detailed drawing of the heart and associated blood vessels and label accordingly. Answer the following questions in your lab notebook.

1. What are the two closed circuits that the heart pumps blood into?
2. Trace the route of the blood circulation through the heart chambers; name each and indicate whether the blood is oxygenated/deoxygenated and high or low pressure.
3. What is the functional purpose of the elastic tissue making up the arch of the aorta? .
4. What are the major valves of the heart? Where are they located, i.e., between what cardiac chambers or intake or exit vessels?
5. What is the function of papillary muscles and chordate tendernae?
6. What and where is the pericardium located? What function does it serve?

B. Cardiac Muscle Tissue (Activity 4, p. 449): Microscopic viewing of slides and illustrations in Text and LM

1) Obtain a longitudinal section of cardiac muscle. Draw or photograph at 400X. Label nuclei and intercalated discs. Measure the length of the cardiac muscle cells and record this next to your drawing. Include a scale bar.

2) Question: Name three ways in which cardiac muscle differs from skeletal muscle.

C. Sheep Heart Dissection: (p. 449)

1. Obtain a preserved sheep heart and dissection implements.
2. Identify heart chambers and major blood vessels including
3. Dissect heart following directions on p 449

4. Photograph or draw dissected heart and include in your lab notebook. Label photograph(s) with anatomical terms on attached sheet.

D. Microscopic Structure of Blood Vessels (Exercise 32, Activity 1, p 472)

1. Obtain a slide showing cross-sectional view of blood vessels. Draw or photograph both artery and vein and include in your lab notebook.

a. Label the three major tunics of each blood vessels and list function

b. Measure the internal lumen using your ocular micrometer and include a scale bar.

b. Write a brief paragraph describing the structural and functional differences between structure of arteries, veins and capillaries.

E. Gross Anatomy of Major Blood Vessels (Activities 3 & 4)

1. Use charts, models, and "Anatomy and Physiology Revealed" CDs to locate and identify major blood vessels (arteries and veins) listed on attached spreadsheet.

2. Draw a diagram tracing the path of arterial blood from the aorta to the femoral and radial arteries.

3. Draw a diagram tracing the path of venous blood from the femoral and radial veins into the vena cavae.

Lab 3: Anatomy of the Heart & Blood Vessels
Anatomy to Know

Heart	Arteries	Veins
Right Atrium	Pulmonary Artery	Vena Cavae (Inferior and Superior)
Left Atrium	Coronary Artery	Pulmonay Vein
Right Ventricle	Aorta (Acending, Decending, and Abdominal)	Left Brachiocephalic Vein
Left Ventricle	Pulmonary Trunk	Right Brachicephalic Vein
Septum	Subclavian Artery	Interior Iliac Vein
Auricle	Brachiocephalic Trunk	External Iliac Vein
Ligamentum Arteriosum	Common Carotid Artery	Femoral Vein
Myocardium	External Carotid Artery	Great Saphenous Vein
Tricuspid Valve (R AV Valve)	Superficial Temporal Artery	Renal Vein
Mitrial Valve (L AV Valve)	Axillary Artery	Suprarenal Vein
Aortic Semilunar Valve	Brachial Artey	Hepatic Veins (L & R)
Pulmonary Semilunar Valve	Radial Artery	Superficial Temporal Vein
Papillary Muscles	Ulnar Artery	External Jugular Vein
Apex	Intercostal Arteries	Internal Jugular Vein
Foramen Ovale	Celiac Trunk	Subclavian Vein
Pericardium	Hepatic Artery	Axillary Vein
Chordae Tendineae	Gastric Arteries	Brachial Vein
	Superior Mesenteric Artery	Cephalic Vein
	Inferior Mesenteric Artery	Basilic Vein
	Common Iliac Artery	Median Cubital Vein
	Renal Artery	Radial Vein
	Femoral Artery	Ulnar Vein
	Vertebral Artery	Azygous Vein
	Middle Cardiac Artery	Ascending Lumbar Vein
	Splenic Artery	Gastric Vein
	Common Hepatic Artery	Inferior Mesenteric Vein
	Mesenteric Artery	Superior Mesenteric Vein
		Middle Cardiac Vein
		Common Iliac Vein

ZOOL 142L

4

Conduction System of the Heart & Electrocardiography

Lab Manual Exercises: Exercise 31 (Activity 1)

Learning Outcomes:

1. List the elements of the intrinsic conduction system of the heart and describe how impulses are initiated.
2. Identify and interpret ECG waves.
3. Calculate heart rate and wave intervals from ECGs recorded during lab period.
4. Evaluate the effects of exercise and body position on ECG waves.

Materials:

	ECG Equipment (2 units)		Padded Mats (5)
	Powerlabs and Laptops (3- optional)		Step-up Benches (2)
	Electrodes (3 per student)		Metronomes
	Electrode Paste		Bicycle or Treadmill
	Cable Leads (2 sets)		ECG Paper (1 roll + 1 pack per class)
	Metric Rulers (10-20)		
	Calipers (5)		
	Alcohol Wipes (Box)		
	ECG Abrasive (Tube)		

Suggested Reading: Exercise 31; Lecture 5; Marieb & Hoehn Ch. 18

A. Intrinsic Conduction System

Examine heart models and figures 31.1 & 31.3 in the lab manual and answer the following questions in your lab notebook.

1. Briefly describe the physiological basis of intrinsic heart beat and autohyrthmicity.
2. What are the extrinsic (coming from outside the heart) and intrinsic (within the heart) control centers for heart rate?
3. List the components of the intrinsic conduction system in sequence starting with the SA-Node.

B. Recording ECGs using a standard ECG Apparatus

(Every student should participate in this exercise)

1. Attach ECG electrodes as directed by your instructor.
2. While sitting up, record 10-20 s of electrical activity. Include this printout in your lab notebook.
3. Label the three recognizable waves that make up a complete ECG wave cycle.
3. Describe or diagram the associated events that are occurring in the cardiac pump at the time of each of these wave deflections (see fig. 31.3)
4. Calculate the average duration of all major waves and segments (see pp. 458-459 for instructions) by measuring **three** ECG tracings. Include these in your lab notebook as Table 1.
5. Estimate the **heart rate** by measuring the distance between two adjacent QRS complexes. Use the equation on p. 460 to estimate heart rate.
5. Compare your results to clinical norms (pp. 466). Are your values normal or abnormal?
6. What would a prolonged P-R segment indicate clinically? What about a prolonged T-R interval?
7. Define the following: Tachycardia, Brandycardia, and Fibrillation

C. Effects of body position and exercise on ECG wave durations and interval

The next activity will compare the effects of body position and exercise on wave intervals and wave height (T wave only). A minimum of 5 students from each lab are needed to participate in this exercise (at least one from each lab group)

1) **Question:** Examine a typical electrocardiogram (figure 31.2). Based on your knowledge of ECG patterns, which wave periods or segments will change (get shorter or longer) during each of the following conditions (lying down, sitting up, breath-hold & exercise).
2) **Hypothesis:**
3) Fill out the subject information in table 2.
4) Hook up the subject to the Power Lab or wireless ECG using leads indicated by your instructor.
5) Record and print 15 s of ECG for each of the 4 conditions following the directions below. Include these in your lab notebook.
6) Using a ruler and the ECG time scale, estimate the duration of each of the wave durations and intervals and record in table 2.
7) Estimate the heart rate from the ECG by measuring the interval between consecutive QRS complexes.
8) Measure the magnitude (height) of T wave in mm and record.
9) Pool waveform data among group members (minimum of 5) and calculate averages for each. Record these in table 2.
10) Construct a bar graph comparing average wave intervals for group members for each of the conditions. Give your figure a title and label all axes.
11) **Discussion and Conclusions:** Write a few paragraphs describing your results. Was your hypothesis supported or refuted? Which wave durations/ intervals changed significantly (from baseline) during each of the activities? Which wave durations remained more-or-less the same? What are the sources of error for your experiment? If you were to redo your experiment, how would you modify the methods? Were there any other correlations with wave/ segment duration and subject information (e.g., age, gender, or fitness)?

Conditions

I. **Lying Down (Baseline):** Have subject lie quietly for 2 minutes before recording 10 s of data. Use this as a baseline to evaluate all other wave patterns.

II. **Sitting Up:** Record 10 s of data immediately after student sits up from a lying position.

III. **Breath Hold:** Have subject hold breath for 30 s. Start ECG at 20 s, and record 10 s more.

IV. **Exercise:** After engaging in 5 min of vigorous exercise (bicycle, treadmill or step-box) have student immediately sit down. Record 10-20 s of data.

Lab Notebook Checklist: Lab 4- Conduction system of the heart
☐ Complete Lab Report

Table 1. Individual ECG Data

Segment		Measured Duration	Normal Duration
P wave	1		
	2		
	3		
	Average		0.06-0.11
P-R Interval	1		
	2		
	3		
	Average		0.12-0.20
P-R Segment	1		
	2		
	3		
	Average		0.08
QRS Complex	1		
	2		
	3		
	Average		<0.12
S-T Segment	1		
	2		
	3		
	Average		0.12
Q-T Interval	1		
	2		
	3		
	Average		0.31-0.41
T wave	1		
	2		
	3		
	Average		0.16
T-R	1		
	2		
	3		
	Average		Variable

Segment		Duration
QRS-QRS	1	
	2	
	3	
Average		

Estimated HR:

Table 2: Effects of Exercise and Body Position on ECG intervals

Subject Info		Cond.	HR	P	P-R int	PR seg	QRS	ST seg	QT int	T	T (HT)	TR
Subject 1		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										
Subject 2		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										
Subject 3		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										
Subject 4		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										
Subject 5		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										
Subject 6		Lying Down (Baseline)										
Gender		Siting Up										
Age		Breath Hold (20 s)										
Fitness (1-5)		Exercise (5 min)										

		HR	P	P-R int	PR seg	QRS	ST seg	QT int	T	T (HT)	TR
Average 6	Lying Down (Baseline)	0	0	0	0	0	0	0	0	0	0
	Siting Up	0	0	0	0	0	0	0	0	0	0
	Breath Hold (20 s)	0	0	0	0	0	0	0	0	0	0
	Exercise (5 min)	0	0	0	0	0	0	0	0	0	0

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6

Human Cardiovascular Physiology: BP & Pulse

Lab Manual Exercises: Exercise 33A (Activities 1-3, 5, 7)

Learning Outcomes:

1. Define systole, diastole, and cardiac cycle.
2. Use a stethoscope to asculate heart sounds.
3. Use a manual sphygmomanometer to measure blood pressure.
4. Palpate major pulse points.
5. Measure or calculate pulse, pulse pressure, mean arterial pressure and stroke volume.
6. Evaluate the effects of exercise and body position on the above measurements.

Materials:

	Automatic Blood Pressure Cuffs (5)		Rulers
	Manual Blood Pressure Cuffs (10)		Calipers
	Student Stethoscopes (10)		PowerLab Units (3)
	Teaching Stethoscopes (2)		Laptop Cart
	Padded Mats (4)		Extra AA Batteries
	Step-Up Boxes		Cable Leads
	Electrodes (20 per class)		Electrode Paste
	Stopwatches		

Suggested Reading: 33A: Lectures 5-6; Marieb & Hoehn Ch. 18-19

A. Auscultating Heart Sounds (Activity 1 p. 493)

Use a stethoscope to listen to heart sounds (S1 and S2) in each of the following locations (see Figure 33a.2 on p 494). (BE SURE TO CLEAN EAR PIECES BEFORE USE)

1) 2nd intercostal space right of sternal margin
2) 2nd intercostal space left of sternal margin
3) 5th intercostal space left of sternum
4) 5th intercostal space right of sternum

Questions:

1) What do the two heart sounds represent (i.e. what *makes* the lub-dup sound you hear in the stethoscope?)
2) Estimate your heart rate.
3) How long is the interval (pause) between the two sounds? What is going on during this pause?
4) Why do we bother listening to heart sounds in the 4 locations described above?

B. Palpating Superficial Pulse Points (Activity 2, p 494)

Palpate each of the following pulse points and estimate the pulse from each.

1) Common Carotid Artery
2) Brachial Artery
3) Radial Artery
4) Posterior Tibial Artery

Question:

1) Which pulse point had the greatest amplitude (which pulse was most easily detected)?
2) Try to palpate the common carotid and posterior tibial arteries simultaneously. Do you notice any difference in pulse between these two points?
3) Now listen to your apical pulse with a stethoscope and palpate your radial artery. Try to measure the lag time between these two events.

C. Using the Powerlab Units to Measure Pulse Waves (Adapted from Act 3 p. 495)

1) Hook up 1-2 subjects in each group to the Powerlab Unit as directed by your instructor.
    a. Attach finger pulse transducer
    b. Attach ECG leads (optional)
2) Record at least 15 seconds of data.
3) Print 1 page (at least 5 s) of representative pulse waves and include in your lab notebook.
4) Measure the amplitude (height) of pulse wave. You may notice that each wave is composed of a tall and short peak. Label these peaks on your printout. What do the two peaks represent?

D. Using a manual sphygmomanometer to measure blood pressure (Activity 5, p 497)

1) Follow the directions in your laboratory manual for obtaining blood pressure readings using a stethoscope and manual sphygmomanometer. Practice measuring BP and pulse with a classmate.
2) Record your individual results under "baseline measurements" in Table 1. Once you are confident with your measurements, move on to "E".

E. Observing the effects of various factors on blood pressure and heart rate (Activity 7, p 500).

1) **Question:** What are the effects of body position on pulse and blood pressure?
2) **Hypothesis:**
3) Obtain readings for each subject for the three scenarios described in the following paragraph.
4) Include all Blood Pressure and Pulse measurements in the Table 1. For each condition, calculate or estimate Pulse Pressure (PP), Mean Arterial Pressure (MAP), and Cardiac Output (CO). Assume a stroke volume of 70 ml/contraction.
3) Compose a bar graph comparing AVERAGE Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and CO for each of the three conditions.
4) Write a paragraph discussing your results.

Conditions for Activity E

Lying Down: Have subject lie quietly for 2 minutes before recording blood pressure. Estimate heart rate by palpating radial pulse for 15 s.

Standing: Measure blood pressure and pulse of subject

a) immediately upon standing.

b) 3 min after standing

F. Effects of Age on Cardiovascular Fitness (Harvard Step Test)**:** In this activity, we will evaluate the effects of age on cardiovascular recovery times (the time it takes the heart to recover resting HR and BP after exercise). In general, people who are more physically fit will have faster recovery times.

Q: How does cardiovascular fitness change with age?

H:

1. Obtain baseline HR & BP data while the subject is seated
2. Have subject stand quietly for 2 minutes.
3. Have the subject step up and down on the step box for 5 minutes (30 steps/minute). The subject's back should remain straight during the exercise. The exercise should be terminated once the subject fails to keep pace for 15 s or feels uncomfortable.
4. Have the subject sit down. Record recovery HR & BP at 1 minute intervals for 3 minutes following exercise.
5. Use this data to calculate subjects index of physical fitness (see pp. 501). Record in Table 2.
6. Graph fitness level vs. age using a scatter graph. Add a trend line if a relationship is evident.
7. Discussion: Write a paragraph discussing your results. Was your hypothesis supported or refuted? What other factors might play a role in cardiovascular fitness?

Table 1: Effects of Body Position on Heart Rate & Blood Pressure

Subject Info		Cond.	HR	Systolic BP	Diastolic BP	Pulse Pressure	Mean Arterial Pressure	Cardiac Output
Subject 1		Sititng (Baseline)						
Gender		Lying Down						
Age		Standing (immediate)						
Est Fitness		Standing (after 3 minutes)						
Subject 2		Sititng (Baseline)						
Gender		Lying Down						
Age		Standing (immediate)						
Est Fitness		Standing (after 3 minutes)						
Subject 3		Sititng (Baseline)						
Gender		Lying Down						
Age		Standing (immediate)						
Est Fitness		Standing (after 3 minutes)						
Subject 4		Sititng (Baseline)						
Gender		Lying Down						
Age		Standing (immediate)						
Est Fitness		Standing (after 3 minutes)						
Subject 5		Sititng (Baseline)						
Gender		Lying Down						
Age		Standing (immediate)						
Est Fitness		Standing (after 3 minutes)						

Table 2. Effects of age on cardiovascular fitness

			Baseline		Interval Following Test								Total Minutes Exercise	Fitness Level
					Immediately		1 min		2 min		3 min			
ID	Gender	Age	BP (sys)	HR	BP (sys)	HR	BP (sys)	HR	BP (sys)	HR	BP (sys)	HR		

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7

Respiratory Anatomy

Lab Manual Exercises: Exercise 36 (Activities 1-3)

Learning Outcomes:

1. Define the following terms: *pulmonary ventilation, external and internal respiration.*
2. Identify the major structures of the respiratory system and describe the functions of each.
3. Identify the tissues comprising the trachea (upper respiratory tract) and lungs (lower respiratory tract) from histological sections.

Materials:

	Laptops (10)		Microscope Accessory Boxes (10)
	Anatomy and Physiology Revealed CDs		Inflatable Pig Lungs (2) and Pump
	Human Torso Model		Respiratory Slides (Lung + Trachea)
	Respiratory Organ Models and Posters		Compound Microscopes (10)
	Bell Jar Lung Models (2)		

Suggested Reading: Exercise 36; Lectures 9; Marieb & Hoehn Ch. 22

A) Gross Anatomy of the Respiratory System (Activity # 1, p.543)

1. Study the anatomy of the upper respiratory system using Anatomy and Physiology Revealed CDs, human torso model, lung models, cat dissection (at option of instructor) and Fig. 36.1 in your lab manual.
2. Identify the structures on the attached "anatomy to know" sheet.
3. Be able to distinguish upper respiratory and lower respiratory structures from one another.

B) Demonstrating Lung Inflation using Inflatable Pig Lung (Activity #2, p 543)

1. Don disposable latex gloves.
2. Demonstrate lung inflation using the inflatable swine lungs.

   Q1: How does the manual inflation of the swine lung model differ from actual inflation of the human lung *in vivo* (in life)?

   Q2: What is this process called? Where does gas exchange take place? Be able to trace the path of inhaled air from the nose to alveolus.
3. Repeat using the bell jar lung model.

   Q: What structures in the human body do the following represent:

Balloons	Rubber Membrane
Y Tube	Plastic Bell Jar

C) Histological Structure of the Respiratory System (Activity 3, p. 543)

a. Obtain each of the following prepared slides:
   i. Esophagus + Trachea
   ii. Normal Lung Tissue
   iii. Smoker's Lung Tissue

b. Examine and photograph using a compound microscope.

c. Be able to identify the following structures:
   A. For trachea, identify **columnar epithelium**, **hyaline cartilage**, **goblet cells, seromucous glands & blood vessels.**
   B. For lung label: **alveoli, alveolar duct, blood vessel, bronchiole**

Lab 7: Anatomy of the Respiratory System
Anatomy to Know

Upper Respiratory System	**Lower Respiratory System**
External Nares	*Conducting Zone*
Nasal Septum	Trachea
Nasal Conchae (Superior, Inferior, & Middle)	Primary Bronchi
Nasal Cavity	Hilum
Nasopharynx	Secondary Bronchi
Uvula	Segmental Bronchi
Oropharynx	Bronchioles
Laryngopharynx	*Respiratory Zone*
Hard Palate	Respiratory Bronchioles (Slide)
Soft Palate	Alveolar Duct (Slide)
Larynx	Alveoli (Slide)
Thyroid Cartilage	*Other Structures*
Circoid Cartilage	Superior Lobe
Epiglottis	Middle Lobe
Hyoid Bone	Inferior Lobe
Vocal Cord	Pleura (Parietal & Visceral)
Blood Vessels	Horizontal Fissure
Pulmonary Artery	Oblique Fissure
Pulmonary Vein	Mediastinum
Pulmonary Trunk	Intercostal Muscles
	Diaphragm
Trachea Histology	**Lung Histology**
Pseudostratified Columnar Epithelium	Bronchiole
Seromucous Gland	Adventitia
Goblet Cell	Lamina Propria
Hyaline Cartilage	Alveolar Duct
Smooth Muscle	Blood Vessel
	Alveoli

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8

Respiratory Physiology

Lab Manual Exercises: Exercise 37A (Activities 1-4)

Learning Outcomes:

1. To define the following:
   Inspiration — Expiratory Reserve Volume (ERV)
   Expiration — Inspiratory Reserve Volume (IRV)
   Tidal Volume (TV) — Minute Respiratory Volume (MRV)
   Vital Capacity (VC)
2. To explain the role of muscles and volume changes in the mechanical process of breathing.
3. To demonstrate proper usage of a spirometer to measure lung volumes.
4. To demonstrate the importance of CO_2 and O_2 on stimulating breathing.
5. To describe bronchial and vesicular breathing sounds.

Materials:

	Tape Measures (10)		Vernier O_2 and CO_2 sensors (2 ea)
	Stethoscopes (10)		Paper Bags or Ziploc Freezer Bags (20)
	Alcohol Pads		Large Respirometer & Vacuum Hose
	Wet Spirometers (2)		Acrylic Respiratory Valve (1)
	Disposable Mouthpieces (25)		Blue Rubber Mouthpieces, Sterilized (10)
	Nose Clips		Sterilizing Solution
	Data Tables		Stopwatches
	Vernier Units (1-2)		Bicycle Ergometer
	Laptops (1-2)		Pulse Oximeter (1)

Suggested Reading: Exercise 37A; Lectures 9; Marieb & Hoehn Ch. 22

A. Respiratory Volumes and Capacities: Refer to Fig. 37A.2 Spirogram

Briefly describe the following Volumes & Capacities and know their NORMAL adult values

Lung Volumes-

Tidal Volume (TV)

Inspiratory Reserve Volume (IRV)

Expiratory Reserve Volume (ERV)

Residual Volume (RV)

Lung Capacities –

Inspiratory Capacity (IC)

Functional Residual Capacity (FRC)

Vital Capacity (VC)

Total Lung Capacity (TLC)

B. Measuring Respiratory Volumes and Capacities using Wet Spirometers (Act 3, p 552)

In this exercise, you will be measuring clinical lung volumes for class members. **Vital Capacity** (VC) is the amount of air that can be voluntarily exhaled from the lungs after a full inhalation.

Q: What effect does height have on Vital Capacity (VC)?

Hypothesis:

1. Measure your height using the meter stick or scale at front of the room.
2. Use the wet spirometer to measure each lung volume three times and record the average.
3. Calculate the capacities and total lung volume (capacities are usually calculated whereas volumes are actually measured)

3) Compare your measured vital capacity against the predicted found in Table 37.A-1 on p. 557

5. Create a scatter graph of height vs. vital capacity for all members of the class. Draw a best-fit line.

6. Write a conclusion paragraph. Was your hypothesis supported or refuted? Did your own lung volumes fall within projected ranges? What were the sources of experimental error?

C. Respiratory Minute Volume: Resting vs. Exercise.

In this exercise, you will be measuring respiratory minute volumes (RMV = # breaths/ min * volume per breath) during rest and exercise.

1. Form a research question & hypothesis using the format below.

Q: What effect does exercise have on MRV?

H: Exercise will (increase/decrease) MRV.

2. Attach the acrylic respirometer valve to the KM meter and apply a nose clip.

3. Breathe easily into the respirometer until you are no longer conscious of the mouthpiece.

4. When you are comfortable, start your stopwatch and record your # of breaths and volume exhaled for 1 minute. Use this data to calculate your **resting MRV.**

5. Repeat while doing a exercise (step-up or bicycle) for 2-min. Begin recording breaths And volumes at the end of the first minute. Use this data to calculate your **exercise MRV**.

6. Graph average MRV (resting vs. exercise) for your group members

7. Write a conclusion paragraph. Was your hypothesis supported or refuted? What were the sources of experimental error?

D. Effects of hyperventilation and re-breathing on breath-hold time

Breathing rate and depth is controlled by the medulla oblongata and pons. Together, they stimulate contraction of the diaphragm and intercostals muscles which ventilate the lungs. The impulse to breathe grows stronger when the blood is low in oxygen and high in carbon dioxide. However, one of these gasses is more important than the other in stimulating an increase in respiration rate. In this activity, we will use breath-hold time as an estimate of motor impulses arising from the brain to the (i.e., short breath-hold time = strong urge to breathe = frequent motor impulses). We will compare breath-hold times between different activities to see which gas has the greatest effect on the urge to breathe. (Urgent: Be sure to wait a minimum of 5 minutes between each trial. Also, be sure to have at least two people available to steady the student should they feel faint).

Q: Which gas, O_2 or CO_2, has the greatest influence on breathing rate?

H:

I) Normal breath hold (Control).

a. Attach the lead of the pulse oximeter to the index finger of the subject. Have them breathe normally until the spO_2 values stabilize.
b. Have subject take a deep breath and hold it for as long as possible. Time w/stopwatch.
c. At break point, record post breath-hold time and spO_2 reading.

II) Hyperventilation followed by breath holding (__CO2__ O2).

a. Execute a 30-second hyperventilation
b. Immediately take a deep breath and hold for as long as possible. Time.
c. At break point, record post breath-hold time and spO_2 reading.

III) Re-breathing into a bag for 60 – seconds (_CO2_O2).

a. Place CO_2 and O_2 Sensors in a new plastic or paper bag.
b. Execute re-breathing into the bag for 60 seconds. Record data and observe changes in CO_2 and O_2 concentrations on Vernier unit.
c. Immediately, take a deep breath and hold it as long as possible. Time.
d. At the break point, record breath-hold time and spO_2 reading.

1) Graph INDIVIDUAL breath hold time and spO_2 readings for each of the three activities as well as the AVERAGE CLASS data.
2) Write a conclusion summarizing your results. Which activity had the longest breath hold time? The shortest? During which activity did the spO_2 drop the lowest? How did ending spO_2 correlate with breath-hold time? Based on these data, which gas do you think is most important in stimulating breathing rate? What additional tests could you do to verify this hypothesis? What were sources of error in your experiment?

Lab Notebook Checklist: Lab 8- Respiratory Physiology (Full Write-Up)

- □ Title
- □ Introduction including Respiratory definitions, Questions, and Hypotheses.
- □ Materials and Methods
- □Lung Volume Data Table
- □ Vital Capacity Graph
- □ MRV Graph
- □ Breath-hold spO_2 graphs
- □ Conclusions: Vital Capacity, MRV, & Breath-hold

Table 1. Effects of height on Vital Capacity & Table 2. Effects of Exercise on MRV						Part B: (Activity 3)					Optional: (Activity 4)		Part C	
						Lung Volumes			Lung Capacities		Forced VC		MRV	
ID	Age	Fitness (1-5)	Sex	HT (cm)	Smoke?	TV	IRV	ERV	IC	VC	FEV	FEV-1	Resting	Exercise

Table 3.	Effects of Hyperventilation and Rebreathing on Breath-Hold Time and spO2							
			Normal		Hyperventilation		Rebreathe	
ID	Gender	Ht (cm)	BH Time	End spO2	BH Time	End spO2	BH Time	End spO2

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9

Anatomy of the Digestive System

Lab Manual Exercises: Exercise 38 and Dissection Exercise # 7

Learning Outcomes:

1) Identify organs of the digestive system
2) Describe the general functions of digestive organs and structures
3) Recognize histological structure of major GI and accessory organs
4) List the enzymes involved in the digestion of proteins, fats, and carbohydrates; state their site or origin, and summarize the environmental conditions promoting their optimal functioning

Materials:

	Dissectible Human Torso (1-2)		Dissection Instruments
	Cats or Fetal Pigs (4-5)		Anatomy & Physiology Revealed CDs
	Gloves (1 pair/student)		Prepared Slides: Liver, salivary glands, stomach & intestine
	Safety Goggles (1 pair/student)		Compound Microscopes

Suggested Reading: Exercise 38; Lectures 10-12; Marieb & Hoehn Ch. 23

A. Anatomy of the Digestive System

Working in teams of 4, dissect a cat or fetal pig following directions from your instructor.

1) Make a sketch of the dissection in your lab notebook
2) Identify major digestive organs and structures (see "Anatomy to Know" sheet).
3) Know the major functions of each organ and the enzymes or secretions produced

B. Histology of the gastrointestinal tract (Exercise 38: Activity 2-4)

Obtain prepared slides of GI tract (esophagus, stomach, or intestine) from your instructor.

1) Make a detailed drawing of a representative cross-section through the GI tract (Use 40x or 100x magnification):
2) Label the 4 layers and summarize their functions
3) Label any distinctive features (e.g., villi or rugae) that might help you distinguish tissue samples taken from different points in the G.I. tract.

C. Histology of accessory organs of digestion

1) Obtain slides of salivary glands, pancreas, & liver
2) Make a drawing of each at 100-400x TM
3) Label major anatomical structures and list digestive functions of each

NOTE: No Write-up is Required for Today's Lab!

Lab 9: Anatomy of the Digestive System
Anatomy to Know

GI Tract	Accessory Organs	Other Structures
Mouth	**Pancreas**	Trachea
Pharynx	**Liver**	Larynx
Esophagus	Cystic Duct	Diaphragm
Tongue	Common Hepatic Duct	Heart
Hard Palate	Gall Bladder	Epiglottis
Soft Palate		Glottis
Epiglottis	**Histology**	Thyroid
Stomach	Salivary Gland	Lungs
Duodenum (small intestine)	1) Duct	Spleen
Jejunum (small intestine)	2) Mucous Cells	Thymus
Ileum (small intestine)	3) Serous Demilunes	
Cardiac Sphincter (Stomach)		
Pyloric Sphincter (Stomach)	Liver	
Ileocecal Valve	1) Lobule	
Caecum (Large Intestine)	2) Connective Tissue Septum	
Ascending Colon (Large Intestine)	3) Central Vein	
Transverse Colon (Large Intestine)		
Descending Colon (Large Intestine)	Pancreas	
Anus	1) Acinar Tissue	
Greater Omentum	2) Pancreatic Islet	
Lesser Omentum		
Greater Curvature	G.I. Tract	
Lesser Curvature	1) Mucosal Layer	
Rugae	2) Submucosa	
	3) Muscularis	
	4) Serosa	
	5) Villi	

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10

Digestive Physiology

Lab Manual Exercises: Exercise 39A Chemical Processes of Digestion

Learning Outcomes:

1) List the enzymes involved in the digestion of proteins, fats, and carbohydrates; state their site or origin, and summarize the environmental conditions promoting their optimal functioning
2) Recognize the variation between different types of enzyme assays
3) Perform chemical tests to determine if digestion of particular macromolecules has occurred.
4) To define, enzyme, catalyst, control, substrate, & product.

Materials: (Based on 4 student groups)

	Hot Plates (4)		Dropper Bottle Lugol's Solution (4)
	250 ml Beakers (4)		Dropper Bottle Benedict's Solution (4)
	Test Tubes (small- 70)		Spot Plates (4)
	Test Tube Racks (12)		Dropper Bottle 1% BPNA (4)
	37° Water Bath (1-2)		Dropper Bottle 1% Trypsin (4)
	Ice Water Bath (1)		Dropper Bottle 1% Pancreatin (4)
	Dropper Bottle 1% Alpha Amylase (4)		Litmus Cream (4)*
	Dropper Bottle 1% Boiled Starch (4)		Vegetable Oil Dropper (4)
	Dropper Bottle 1% Maltose (4)		Bile Salts
	Parafilm		Safety Goggles
	Lab Quest w/ pH Sensor (1)		Dropper Bottle 0.1 N HCL (4)

***** Fresh Cream to which powdered litmus is added until deep blue

Suggested Reading:

Lab Manual Exercise 39A

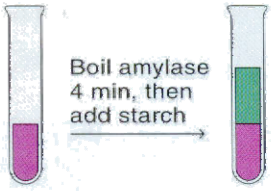
Assigned Tasks:

A. Assessing Starch Digestion by Salivary Amylase (Exercise 39A: Activity
1) Follow the directions for Activity 1 (p 598)
2) Form a hypothesis as to which tube(s) will show successful starch digestion
3) Include a table of your results (see table on p 600) in your lab notebook
4) Include a brief paragraph discussing your results. Why did starch digestion occur in some tubes but not others? What effect did temperature have on rate of enzyme digestion?

B. Assessing Protein Digestion by Trypsin (Exercise 39: Activity 2)
1) Follow the directions for Activity 2 (p 600-601)
2) Form a hypothesis as to which tube(s) will show successful protein digestion
3) Include a table of your results (see table on p 601) in your lab notebook
4) Include a brief paragraph discussing your results. Why did protein digestion occur in some tubes but not others? What effect did temperature have on rate of enzyme digestion?

C. Demonstrating the emulsification action of bile and assessing fat digestion by lipase (Exercise 39A: Activity 3)
1) Follow the directions for Activity 3 (p 602)
2) Form a hypothesis as to which tube(s) will show successful lipid digestion
3) Include a table of your results (see table on p 602) in your lab notebook
4) Include a brief paragraph discussing your results. Why did lipid digestion occur in some tubes but not others? What effect did bile have on lipid digestion? What effect did temperature have on rate of enzyme digestion?

Salivary Amylase Digestion of Starch

Tube no.	1A	2A	3A	4A	5A
Additives (3 gtt ea)					
Incubation condition	37°C	37°C	37°C	37°C	37°C
IKI test (color change)					
Positive (+) or negative (−) result					
Benedict's test (color change)					
Positive (+) or negative (−) result					

Additive key:

= Amylase = Starch = Maltose = Water

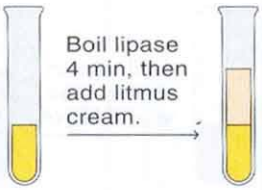
Trypsin Digestion of Protein

Tube no.	1T	2T	3T	4T	5T
Additives (3 gtt ea)					
Incubation condition	37°C	37°C	37°C	37°C	0°C
Color change					
Positive (+) or negative (−) result					

Additive key:

= Trypsin = BAPNA = Water

Pancreatic Lipase Digestion of Fats

Tube no.	1L	2L	3L	4L	5L	4B	5B
Additives (5 gtt ea)							
Incubation condition	37°C	37°C	37°C	37°C	0°C	37°C	0°C
Color change							
Positive (+) or negative (−) result							

Additive key:

= Lipase = Litmus cream = Water = Pinch bile salts

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11

Urinary Anatomy

Lab Manual Exercises: Exercise 40 (Activities 1-3)

Learning Outcomes:

1) To describe the function of the urinary system.
2) To identify, on an appropriate diagram or torso model, the urinary system organs and to describe the general function of each.
3) To compare the course and length of the urethra in males and females.
4) To identify major anatomical structures within the kidney.
5) To trace the blood supply of the kidney from the renal artery to the renal vein
6) To define glomerular filtration, tubular reabsorption, and tubular secretion, and to identify the nephron areas involved in these processes
7) To define micturition, and to explain pertinent differences in the control of two bladder spincters (internal and external).
8) To recognize structures from histological sections of kidney and bladder.

Materials:

	Human Torso Models (2)		Compound Microscopes
	Kidney Models (2-3)		Kidney Slides (10)
	Bladder Model (1)		Urinary Bladder Slides (5)
	Cat or Pig Kidneys (can be from dissected specimens- 5)		Safety Glasses
	Disposable Gloves		

Suggested Reading:

Lab Manual Exercise 40

A. Activity #1: Identifying Urinary System Organs

1) Examine the cat dissection, kidney model, and urinary bladder model.

2) Sketch (or photograph) and label major anatomical features (see list below) and list the function of each:

renal capsule
urinary bladder
ureter
renal vein & artery
cortex
medullary region
renal pyramids
papilla or apex
renal columns
renal pelvis
hilus
urethra
major calyces
minor calyces
segmental arteries
interlobar arteries
arcuate arteries

B. Activity #2A – Nephron Anatomy

1) Structure of the Nephron: Draw a diagram of a nephron and label the following terms AND indicate their function(s).
2) Indicate the locations where **filtration, secretion, & reabsorption** take place

Glomerulus*
Renal tubule*
Bowman's Capsule*
Podocytyes
Renal Corpuscle
Interlobular artery
Afferent arterioles
Eferrent arterioles
Proximal conv. tubule
Loop of Henle
Distal convoluted tubule
Collecting ducts

C. Activity # 2B: Kidney Histology

1) Examine a prepared slide of kidney tissue at high power (400x)

2) Draw or photograph a representative section and identify and label terms with "*" above.

D. Activity # 2C: Bladder Histology

1) Examine a prepared slide of urinary bladder at scanning (40X) or low power (100X)

2) Draw a representative cross-section through the bladder and label the three major layers (muscuaris, mocosa, and Adventitia)

3) List the tissue that composes each layer

4) List the function of each layer.

5) Answer the following questions about bladder function:

1. What is micturition?
2. Identify the internal and external sphincter.
3. What purpose do stretch receptors serve?
4. Does the bladder have skeletal or smooth muscle?
5. Describe the micturition reflex; volume of urine that will trigger reflex?
6. Cause[s] of incontinence?

Lab Notebook Checklist: Lab# 11- Urinary Anatomy

No lab write-up is required, however, you will be expected to correctly identify the indicated anatomy and answer the above questions for the lab practicum

ZOOL 142L

12

Urinalysis

Learning Outcomes:

1. Classify odor, color and clarity of urine.
2. Screen urine for abnormal organic components using reagent test strips.
3. Determine specific gravity of urine using refractometer.
4. Identify common components of urine sediments.
5. Describe the effects of various substances on urine volume, pH and specific gravity.

Materials:

	Urine Samples		Latex Gloves (20 pairs)
	Refractometers (10)		DiaScreen ® Reagent Strips (40)
	15-ml Centrifuge Collection Tubes (40)		Pipettes
	Urinalysis Recording Data Sheets (20)		Laboratory Request Forms
	E.R.D. Kits (10)		Distilled Water
	Timers (10)		*Urine Collection Cups (22)
	Microscopes w/ ocular micrometers (10)		Multi-speed Centrifuge (2)
	Microscope Slides & Coverslips		Black Permanent Markers (10)
	Urine Sediment Stain		Parafilm
	Kimwipes® or Bibulous Paper		Biohazard Bag and Sharps Container
	12 Cans Diet Soda		10 Bottles (500 ml) distilled water
	Drinking Cups		Baking Soda and measuring spoon
	Salted Pretzels (6 servings)		

*Students are to bring an animal urine sample to class, should be fresh or refrigerated if stored over night.

Suggested Reading:

Martini-Johnson, L. Chapter 5, Urinalysis. In Hendrix, M. and M. Sirois (eds): *Laboratory procedures for veterinary technicians,* 5th ed, St Louis, 2007, Mosby.

Zinkl, J.G. Chapter 18, Urinary Sediment and the Cytology of the Urinary Tract. In Cowell, R.L., R.D. Tyler, J.H. Meinkoth, and D.B. DeNicola (eds): *Diagnostic cytology and hematology of the dog and cat,* 3rd ed, St Louis, 2008, Mosby.

EXERCISE 12: Urinalysis

Figure 5.1 Equipment and supplies necessary for urinalysis. Necessary equipment includes a multi-speed centrifuge, compound microscope, refractometer and urine hydrometer. Necessary supplies include: urine collection cups, 15 ml centrifuge tubes, DiaScreen® test strips, microscope slides, coverslips, parafilm, urine sediment stain, urinalysis data sheets, latex gloves, safety goggles, biohazard container and ERD kit (not shown).

Introduction

The urinary system consists of the **kidneys, ureters, urinary bladder,** and **urethra.** The main functions of the urinary system are to : 1) filter the blood 2) maintain proper water and ion balance and 3) excrete metabolic waste products 4) maintain blood pH. **Urinalysis** is the examination of urine to assess proper renal (kidney) function. Because the urinary and circulatory systems are directly linked, urinalysis can also be used to screen for blood and endocrine disorders and to asses metabolic function.

In this lab you will evaluate the physical and chemical properties of two urine using standard lab techniques. The first sample will be a **standard** supplied by your instructor, who has already performed a baseline urinalysis on the urine. You will record the results of your urinalysis for this sample in table 5.1. Once your analysis is complete, you should compare your results to those obtained by your instructor. Note any major discrepancies, as these may indicate flawed technique, contamination, or incorrect interpretation or the results. For the second sample (**unknown**), you should be obtain a fresh sample from the family cat or dog. If an animal is unavailable, you can use your own urine for analysis. Record the results for the unknown sample in table 5.2.

[Type text]

Because urine may contain potentially infectious organisms, it is imperative that you observe all lab safety procedures and wear personal protection (gloves, safety glasses, and a lab coat) at all times. Dispose of all materials coming in direct contact with urine (e.g., pipetts and centrifuge tubes) in a biohazard container or bag. **Contamination** is one of the greatest sources of error in analyzing urine samples. Be sure to observe **sterile technique** at all times; do not re-use microscope slides, centrifuge tubes or pipettes and don a fresh pair of gloves when changing samples.

The Nephron

In order to correctly interpret the results of urinalysis, it is essential that you understand the basics of kidney function. Each mammalian kidney consists of approximately 1 million microscopic structures called **nephrons** (Figure 5.2). The nephron consists of two main parts: the glomerulus and the renal tubule. The **glomerulus** is a small capillary bed. The glomerulus is supplied with blood via the afferent (= towards) arteriole and drained by the efferent (= away) arteriole. Surrounding the glomerulus is a funnel-shaped receptacle known as the **Bowman's capsule**, which is the entrance to the hollow **renal tubule**. The renal tubule snakes its way in and out of the kidney

interior and eventually empties into a **collecting duct** which eventually drains into the **renal pelvis**. The nephron has three main functions: filtration, reabsorption and secretion.

Filtration

Like most capillary beds, the glomerulus consists of a single layer of epithelial cells. Unlike most other capillary beds, however, the glomerulus is under moderately high blood pressure. These two features make the glomerulus *extremely* leaky. Essentially, the glomerulus acts as a microscopic colander: Small molecules (e.g., water, salts, urea and glucose) pass easily through the glomerular membrane (**filtration**) while plasma proteins and blood cells, which are comparitively large, remain in the blood and are shuttled away from the glomeurulus via the efferent arteriole.

Reabsorption

The filtrate that leaks out of the glomerulus enters the Bowman's capsule and flows through the renal tubule. It is important to point out that anything that remains in the renal tubule is destined to be excreted in the urine. Although the filtrate does contain lots of "unwanted" solutes, it also contains a good deal of materials that we want to hang on to, namely water, sodium and glucose. Fortunately, the renal tubule contains solute-specific membrane proteins (a sort of "cellular check-out" lane) which allow for selective **reabsorption** of these "desirable" substances from the tubule back into the bloodstream. Under normal circumstances, the number of "check-out" lanes is sufficient to completely reabsorb all of the filtered glucose back into the blood. If, however, the blood glucose levels are excessively high, as might occur in a patient with **diabetes mellitus**, the check-out proteins become swamped, and some of the glucose ends up being excreted in the urine.

Secretion

The final job of the nephron is to get rid of any "undesireable" solutes that remain in the blood. During secretion, these solutes (e.g, urea) are shuttled from the capillaries into the renal tubule and are excreted in the urine.

Reading Comprehension:

1) What are the three main functions of a nephron? In which direction do solutes move?

2) Which components of the filtrate are actively reabsorbed?

3) **Proteinuria** is an abnormal condition whereby plasma proteins (e.g., albumin) end up being excreted in the urine. Speculate as to which of the three nephron functions are impaired in a patient with poteinuria.

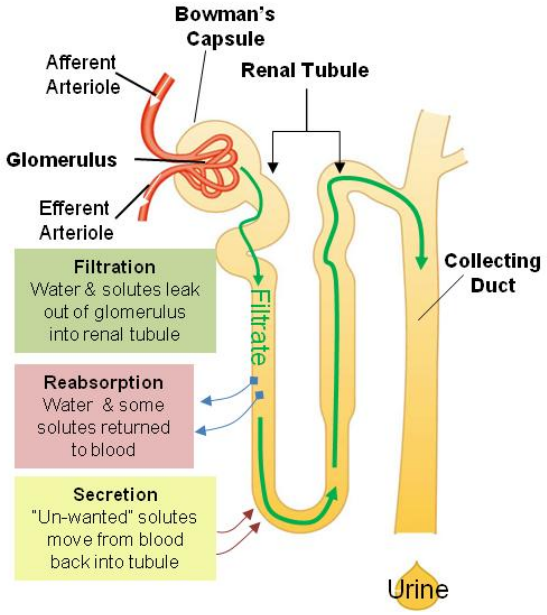

Figure 5.2 A simplified diagram of nephron anatomy and function

Physical Properties of Urine

Although we will be using quantitative (numerical) tests of urine composition in future activities, a qualitative (subjective) assessment of urine color and clarity can still tell us a lot about the hydration and health of an animal. Urine consists mainly of water, **solutes** (anything dissolved in urine) and suspended sediments. Most of these components are colorless under normal conditions. One exception are pigments called **urochromes**. Urochromes are derived from the metabolic breakdown of **hemoglobin** when worn-out erythrocytes (red blood cells) are destroyed and recycled in the spleen. It is these pigments that give urine its characteristic yellow color. In a healthy animal, the *production* of urochromes (and most other solutes) remains relatively constant and does not vary with water consumption. Thus the coloration and hue of urine can often be used as an indicator of its

solute concentration. A dark-yellow coloration typically indicates concentrated urine (high concentration of dissolved solutes). The ability of an animal to produce dark, concentrated urine is often considered a "good" sign, indicating that the renal tubule is doing its job of re-absorbing water efficiently (Very dark urine can be a result of dehydration). Conversely, a clear or straw-coloration suggests dilute urine with low solute-to-water ratio. This may result from excessive water intake or inability of the renal tubule to reabsorb water efficiently. Urine coloration can also be affected by diet and drugs. For these reasons, solute concentration should be verified by measuring the specific gravity with a refractometer (Activity 2).

The **clarity** or transparency of urine can be affected by the presence of **mucus** or suspended **sediments** (e.g., urine crystals, blood cells, and casts) or **lipids** (fats) in the urine. The clarity of urine is typically evaluated by holding a vial of fresh sample up to 12 pt. newsprint. If the text can be read without distortion, it is termed ***clear***. If the text cannot be read clearly, the sample is termed ***hazy, cloudy,*** or ***turbid***, depending on the degree of opacity. In healthy cats and dogs, the urine should be clear to hazy. A turbid sample may indicate the presence of excessive crystals, mucus, or blood cells. Alternatively, excessive turbidity may indicate that the sample has begun to **ferment** due to bacterial growth in the urine. It is for this reason that urine should always be analyzed within 30 minutes of collection. If this is not possible, the urine may be refrigerated (maximum of 6-12 hrs) or preserved with formalin.

The **odor** of urine is typically of little diagnostic use. *Fresh* cat or dog urine usually has a subdued odor with little ammonia smell. Some foods can impart a distinct odor on the urine (ever smelled your pee after eating asparagus?). A ***fruity*** odor may sometimes indicate the presence of ketones, which are generated during fat metabolism. *Excessive* **ammonia** smell may occasionally indicate a bacterial infection of the urinary tract, but more often indicates that the sample has been left out too long before analysis.

Figure 5.3 Color variation in urine samples typically reflect differences in solute concentration**.** Both of the above samples were voided (urinated) from the same animal. The left sample was collected in the early morning. The dark-yellow coloration indicates a high concentration of dissolved solutes (SpG. 1.020). The right sample, collected in the early evening, is more dilute thus and contains a lower concentration of solutes (SpG. 1.010).

ACTIVITY 1

Classifying the color, odor, and clarity of urine

1) Don latex gloves and safety glasses.
2) Collect 10 ml of fresh urine and place into 15 ml centrifuge tube.
3) Hold tube up to the light and classify the color of the sample according to the criteria in table 5.1.
4) Hold the sample up to 12 pt newsprint.
5) Classify the clarity according to the criteria in table 5.1.
6) Wave your hand over the open sample tube and characterize the odor using table 5.1.
7) Record the results in Table 5.1 (standard) or 5.2 (unkown).

Figure 5.4 Using the refractometer to determine the specific gravity of urine. A) Apply a large drop of fresh urine to the prism on the refractometer and close the plastic cover over the prism. Take care that no air bubbles are trapped under the cover, as this can result in errant measurements. B) Hold the refractometer under a bright light and look through the eyepiece. C) Read the specific gravity from the appropriate scale. (The specific gravity of the sample in the photo is 1.020).

Specific Gravity

Although urine coloration can give us a qualitative estimate of solute concentration in urine, we should always quantify the concentration by measuring the specific gravity. The **specific gravity** is a measurement of the **density** (mass/volume) of a solution. The specific gravity of a solution increases with the amount of solutes dissolved in it. Distilled water, which lacks solutes, has a specific gravity (abbreviated SpG) of 1.000, meaning that 1 ml of distilled water weighs exactly 1 gram. Seawater, which contains water and dissolved salts, has a higher specific gravity (around 1.020).

The specific gravity of urine can vary considerably, depending on the amount of water consumed by the and the degree of water loss from sweating (humans) or panting (cats and dogs). Typical values for common domestic species are listed in *Laboratory Procedures for Veterinary Technicians* Table 5-3 (p. 158).

The specific gravity of urine is typically measured with a **refractometer** (Figure 5.4). The refractometer is a telescope-like instrument consisting of a wedge-shaped prism connected to a small lens by a short length of tube. The refractometer measures the refractive index (ability to bend light) of a solution. Because solute-laden liquids bend light rays more than dilute solutions, we can use this refractive index to estimate the specific gravity. Most refractometers contain multiple scales for measuring the density of various body fluids (e.g., blood serum and urine). Always be sure you are using the SpG. scale to measure the density of urine.

ACTIVITY 2

Measuring the Specific Gravity using a Refractometer

1. Don latex gloves.
2. Place a drop of urine onto the center of the refractometer prism (Figure 5.4).
3. Gently close the prism cover over the urine droplet.
4. While standing under bright light, hold the refractometer up to your eye and focus by turning the eyepiece.
5. Read the specific gravity from the appropriate scale (marked SpG) at the point where the blue and white horizons meet.
6. Record your results in table 5.1 (standard) or 5.2 (unknown).
7. Gently clean the prism with an alcohol pad or kimwipe. Allow to prism dry before replacing the cover.

EXERCISE 12: Urinalysis

Chemical Properties of Urine

Mammalian urine can contain a veritable slew of organic and inorganic solutes. Knowing the concentrations of these solutes can tell us a great deal about the metabolic state of the animal and help us to evaluate the function of the circulatory, digestive, urinary and endocrines systems. The relative concentrations of these chemicals are typically measured using multi-panel test strips (Figure 5.5). Each panel contains a unique chemical reagent which will change color when exposed to its respective solute. The degree of color change often indicates the relative or absolute concentrations of the particular solute. A word of caution about the use of test strips; the results of the test can be affected by the specific gravity and age (freshness) of the sample as well as the age and storage condition of the reagent strips (check expiration dates and always re-cap the bottle!). In addition, small amounts of reagents can sometimes be transferred from one panel to another if the stick is dipped into the urine, thus causing incorrect results. For this reason, you should always pipette individual droplets of urine to each square and never let you pipette touch the reagent panel!

For this lab, you will be using DiaScreen® 10 strips, so named because they measure 10 different urine components. A brief description of each component is listed below. Typical values for each are listed in *Laboratory Procedures for Veterinary Technicians* Table 5-3 (p. 158).

Specific Gravity: If you have ever used a "mood ring" to estimate your spouse's demeanor, they you will have an idea of how accurate reagent strips are at measuring specific gravity. Disregard the value on the test strip and measure the specific gravity using a refractometer (Activity 2).

pH: The pH scale measures the relative concentration of **hydrogen ions** (H+) and **hydroxide ions** (OH-) in a solution. The pH scale ranges from 0 to 14. Solutions with an equal concentration of H+ and OH- ions (e.g., distilled water) have a pH of 7 and are termed **neutral**. Solutions which contain more OH- than H+ have pH values *greater* than seven and are termed **basic** or alkaline. The greater the amount of OH-, the higher the number. Solutions with more H+ than OH- have pH values *less* than seven and are termed **acidic**. It is important to point out that the pH scale is a **log scale**, meaning that the H+ and OH- concentrations change *ten-fold* for each step in the scale. One of the primary functions of the kidneys is to maintain a relatively constant blood pH by excreting excess H+ or OH- in the urine. This can cause urine pH to fluctuate depending on diet and metabolic state. That said, the normal pH for canine and feline ranges between 7-8.

Blood: The DiaScreen® strips test for both whole (non-hemolyzed) as well as ruptured (hemolyzed) erythrocytes. Whole erythrocytes are much too large to pass through the glomerulus, and thus are not normally part of the glomerular filtrate. Presence of non-hemolyzed blood in the urine (hematuria) is more often a sign of bleeding down-stream of the glomerulus (e.g., in the ureters, urethra or urinary bladder). In contrast, presence of hemolyzed blood in the urine (hemoglobinuria) may indicate lysis (rupture) of blood cells within the systemic blood vessels.

Leukocytes: Leukocytes (white blood cells) are occasionally present in the urine of menstruating animals (due to contamination of the urine) or those with infections of the lower urinary-tract. However, this is another case where the reagent strips fall short. False-positives are common in all animal species, especially cats. Verify all positive results by searching for intact leukocytes in the urine sediment (Activity 4).

Nitrite: Nitrites (NO2-) are abnormal components of urine which are formed by bacterial decomposition of urinary **nitrates.** When present, they suggest a bacterial infection of the urinary tract (rare in cats and dogs) or contamination and improper storage of the sample.

Protein: Whole blood contains several types of plasma proteins including albumin, fibrinogen, immunoglobulins (antibodies). With a few

exceptions, most plasma proteins are too large to pass through the glomerulus. Presence of plasma proteins in urine (**proteinuria**) may indicate damage to the glomerular membrane and the onset of kidney disease. Some mammals (e.g., humans) may exhibit temporary, non-pathological (not due to illness) proteinuria as a result of pregnancy, vigorous exercise, or even prolonged periods of standing (less-common in the dog and cat). Finally, despite what it says on the bottle, most "protein" test panels will only detect albumin, and only in fairly large amounts.

If renal disease is suspected, the urine may also be screened with an **ERD** kit (Early Detection of Renal Disease; Activity 4). The ERD test can detect **microalbuminaria** (presence of small amounts of albumin) at levels down to.

Bilirubin: Bilirubin is a bile pigment derived from the normal recycling of erythrocytes in the spleen. Like other hemoglobin by-products (e.g. urochromes and urobilogen), trace amounts of bilirubin may occasionally be found in the urine of the cat and dog. Large amounts of bilrubin in the urine may be an indicator of liver disease.

Ketones: Presence of large amounts of ketones (**ketonuria**) is abnormal in humans and most domestic animals and indicates that the body is relying almost exclusively on metabolism of fats to meet its metabolic needs. This can occur when an animal is starved or is on a high-fat/low-carbohydrate diet (think Atkin's Diet). Ketonuria in a well-fed animal may be a sign of *diabetes mellitus*.

Glucose: Glucose is a monosaccharide (simple sugar) which is always present in blood plasma. Although glucose is small enough to be filtered through the glomerulus, it is normally completely reabsorbed into the renal tubule and thus is not a normal component of urine. However, if blood glucose levels are severely elevated (as with a diabetic animal), there may be insufficient membrane receptors to re-absorb all of the glucose, and some will be excreted in the urine (glucosuria). A side-effect of glucosuria is that renal absorption of water is impaired, causing more water to be excreted in the urine (which will have a lower than normal SpG). ***Diabetes mellitus*** literally means "increased quantities of sweet urine" and was once confirmed by tasting the urine of a suspect animal or person (aren't you glad they make glucose test strips?).

Urobilogen: Urobilogen (yet another by-product of hemoglobin recycling) is produced from bilirubin in the small intestine and is normally present in urine in small amounts. Excessive levels of urobilogen may indicate liver disease.

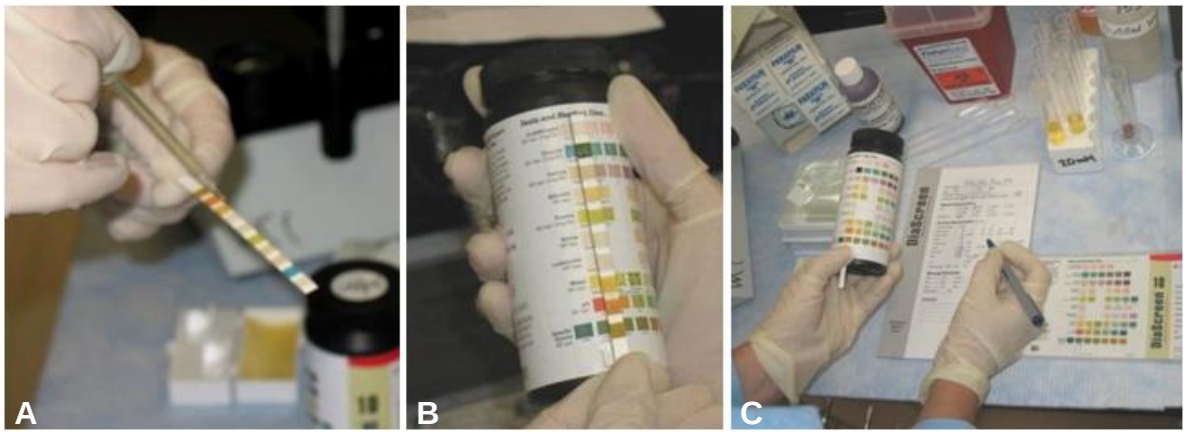

Figure 5.5 Use of DiaScreen® test strips to identify abnormal components of urine. A) Apply individual urine droplets to each square of the test strip. Care should be taken not to splash urine from one square to another, as this can result in incorrect test results. B) Compare the test strip to the color chart on the back of the test strip container. Be aware that some squares should be read at 60 or 120s after exposure. C) Record the results of the DiaScreen® test on the appropriate data sheet or table.

ACTIVITY 3

Using Reagent Strips

1. Don latex gloves.
2. Collect 10 cc of urine and place into 15 ml centrifuge collection tube.
3. Select 1 Reagent Strip from container.
4. Using pipette place 1 drop of urine onto each cotton swab on reagent strip.
5. Leave pipette in tube and set aside.
6. Gently tap off excess urine from reagent strip.
7. Note “Reading Times” on reagent container for obtaining results from reagent strip.
8. Hold reagent strip against container and line up swabs for reading results.
9. Record results at appropriate times in Table 5.1 (Standard) or 5.2 (Unknown).

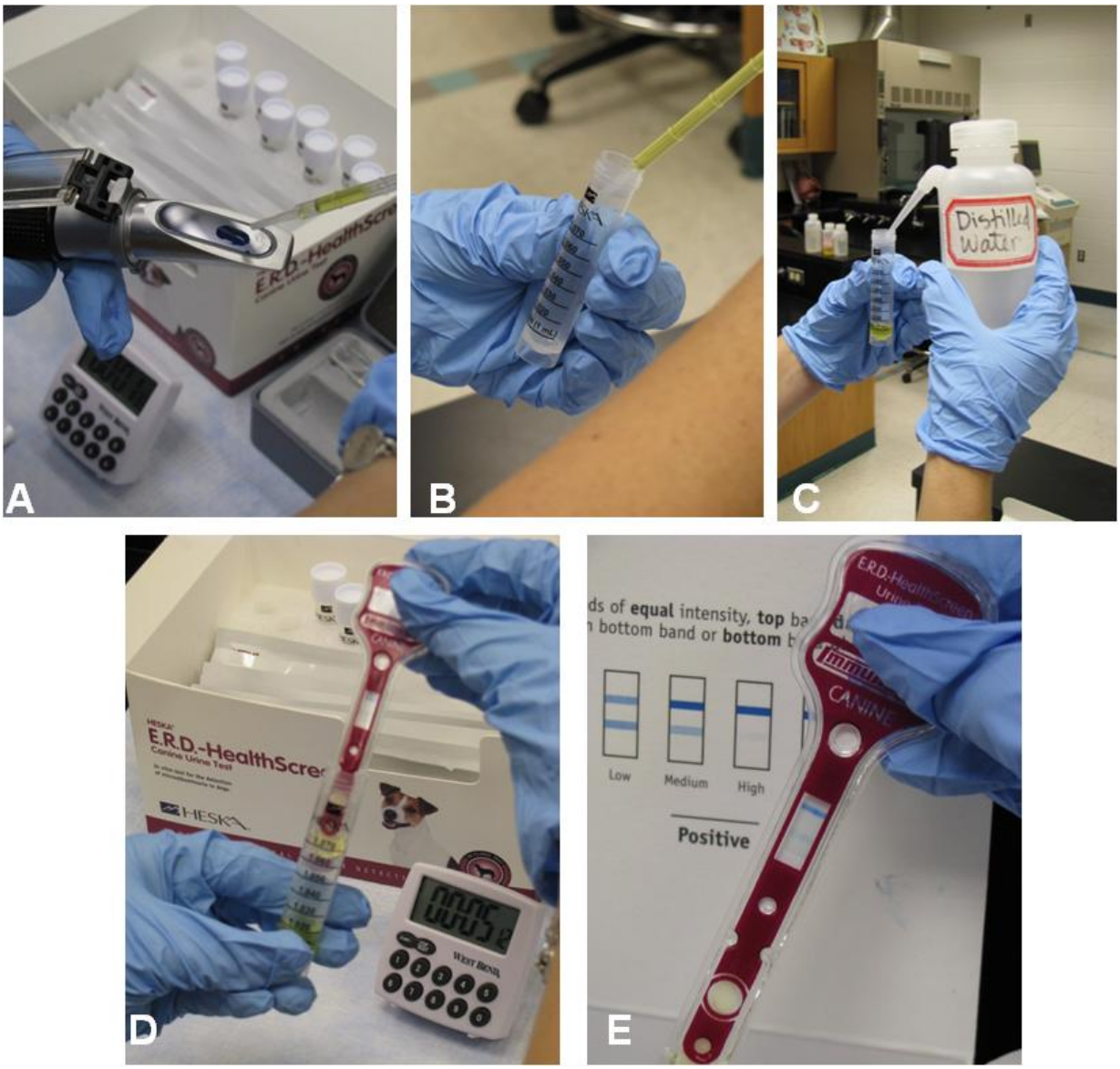

Figure 5.6 Using an ERD kit to screen for microalbuminaria. A) Measure the specific gravity of the urine using a refractometer. B) Pipette the required amount of urine into the sample tube C) Dilute to the appropriate level with distilled water. Thoroughly mix the sample by inverting the tube 2-3 times. D) Immerse the ERD stick in the urine sample and wait 10 minutes. E) Read test by comparing to the color band key on inside of kit (the test shown indicates a high positive test for albumin).

ACTIVITY 4

Screening for Early Renal Disease

1. Don latex gloves.
2. Collect 5 cc of urine and place in 15 ml centrifuge tube.
3. Select 1 E.R.D. test kit.
4. Follow instructions for obtaining USG (urine specific gravity).
5. Follow kits instructions after obtaining USG.
6. If USG is 1.020 or less add sample to the line marked 1.020 line.
7. If USG is above 1.021 to 1.040 then add the sample to the line marked sample and add distilled water to the line that matches the specific gravity of the sample.
8. If USG is greater than 1.040 dilute the 50:50, use 1ml of urine with 1ml of distilled water and re-measure the USG. This is the specific gravity utilized for running the test.
9. Place lid on tube and invert 3-4 times, mixing the urine sample.
10. Open ERD package and place test strip into mixed sample 3-4 times, allow to sit.

11. Set timer for 10 minutes, return and record results in Table 5.1 (Standard) or 5.2 (Unknown).

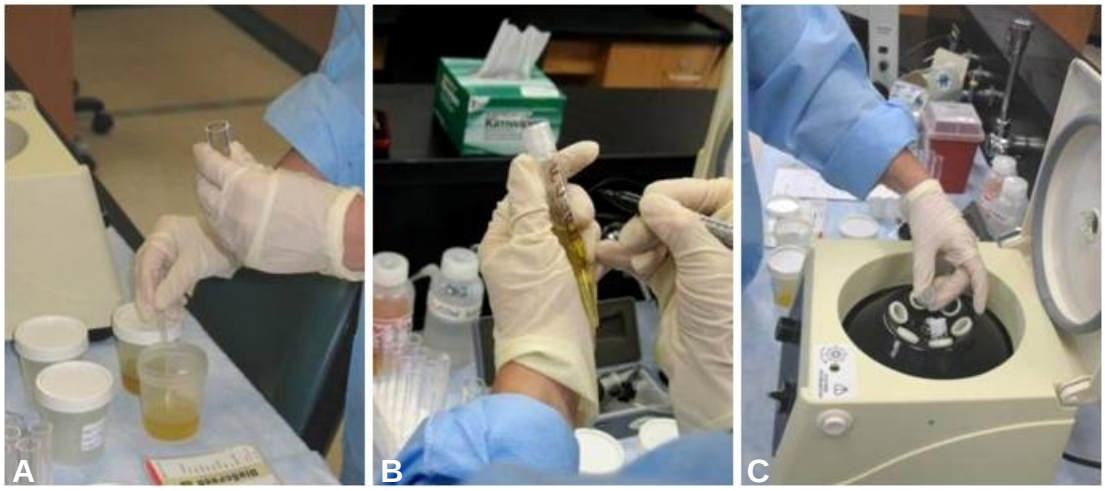

Figure 5.7 Preparation of a urine sample for centrifugation. A) Pipette 10 ml of urine into a 15 ml centrifuge tube. B) Cover with parafilm and label. C) Place the centrifuge tube(s) into the centrifuge and spin for 3-5 minutes at the appropriate speed. Be sure to counter-balance samples so the centrifuge is properly balanced. If spinning a single sample, place a blank tube with an equivalent volume of water opposite of the sample.

ACTIVITY 5

Microscopic Analysis of Urine Sediment,

1) Don latex gloves
2) Using pipette collect 10 cc from sample provided and place into 15-ml centrifuge collection tube. Label tube for identification with black marker.
3) Place centrifuge tube in centrifuge using counter weight for sample's volume. Can be another sample or can use plain water with same volume.
4) Close lid and set centrifuge on urine speed. Set timer for 3-5 minutes.
5) Gently remove sample(s) from centrifuge and set aside.
6) Gently take your sample to the laboratory sink and in one motion invert tube to pour out urine.
7) Return tube to the upright position and gently flick the remaining sample/sediment with your finger to mix together.
8) Using your pipette remove small amount of urine from tube and place 1 drop on left side of a microscope slide and 1 drop on right side of slide. Return pipette to centrifuge tube and set aside.

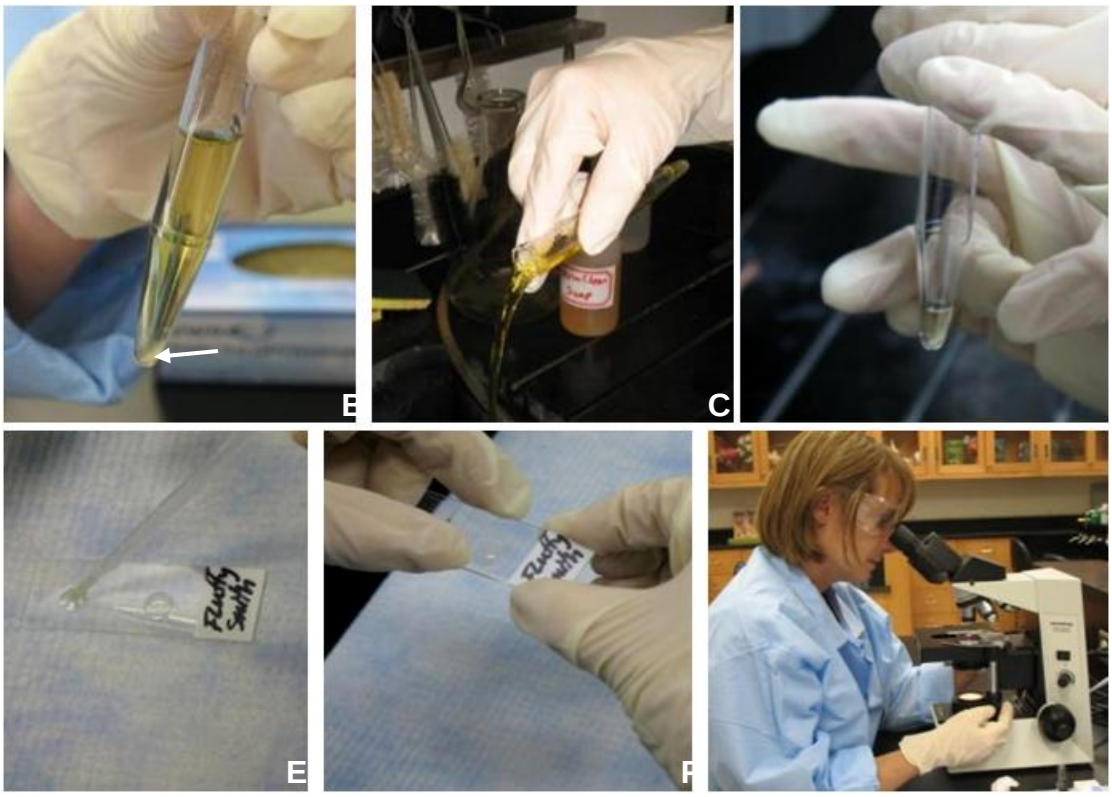

Figure 5.8 Procedures for extracting sediment from a centrifuged sample. A) Remove the sample from the centrifuge and carefully remove the stopper or parafilm. The sediment should be visible as a solid pellet at the bottom of the centrifuge tube (arrow). B) Pour out the liquid component of the urine by inverting the tube. C) Re-suspend the sediment in the remaining liquid by flicking vigorously. D) Pipette two drops onto a clean microscope slide. Stain one of the samples with sediment stain (not shown). E) Carefully coverslip each of the droplets. Remove any excess fluid with a Kimwipe or bibulous paper. F) Observe both samples under the microscope.

9. Place 1 cover slip on each sample.
10. Place slide on microscope stage and focus on 1 sample using 4x objective.
11. Next rotate to 10x objective (Low Power) and focus, and scan entire slide from left to right starting in one of four corners. Take note of any significant findings and write down stage measurements,
12. Rotate microscope to 40x objective (High Power) and focus, scan 10 to 15 fields and note any significant findings. Refer to you Crystal Identification Chart or Atlas of Urinary Sediment textbook.
13. Return to 4x object. Apply 1 drop of Sediment Stain to the second sample from the side of the cover slip allowing the stain to seep and mix with the sample.
14. Repeat Steps 13-15.
15. Record your data in Table 5.1 (Standard) or 5.2 (Unknown). .

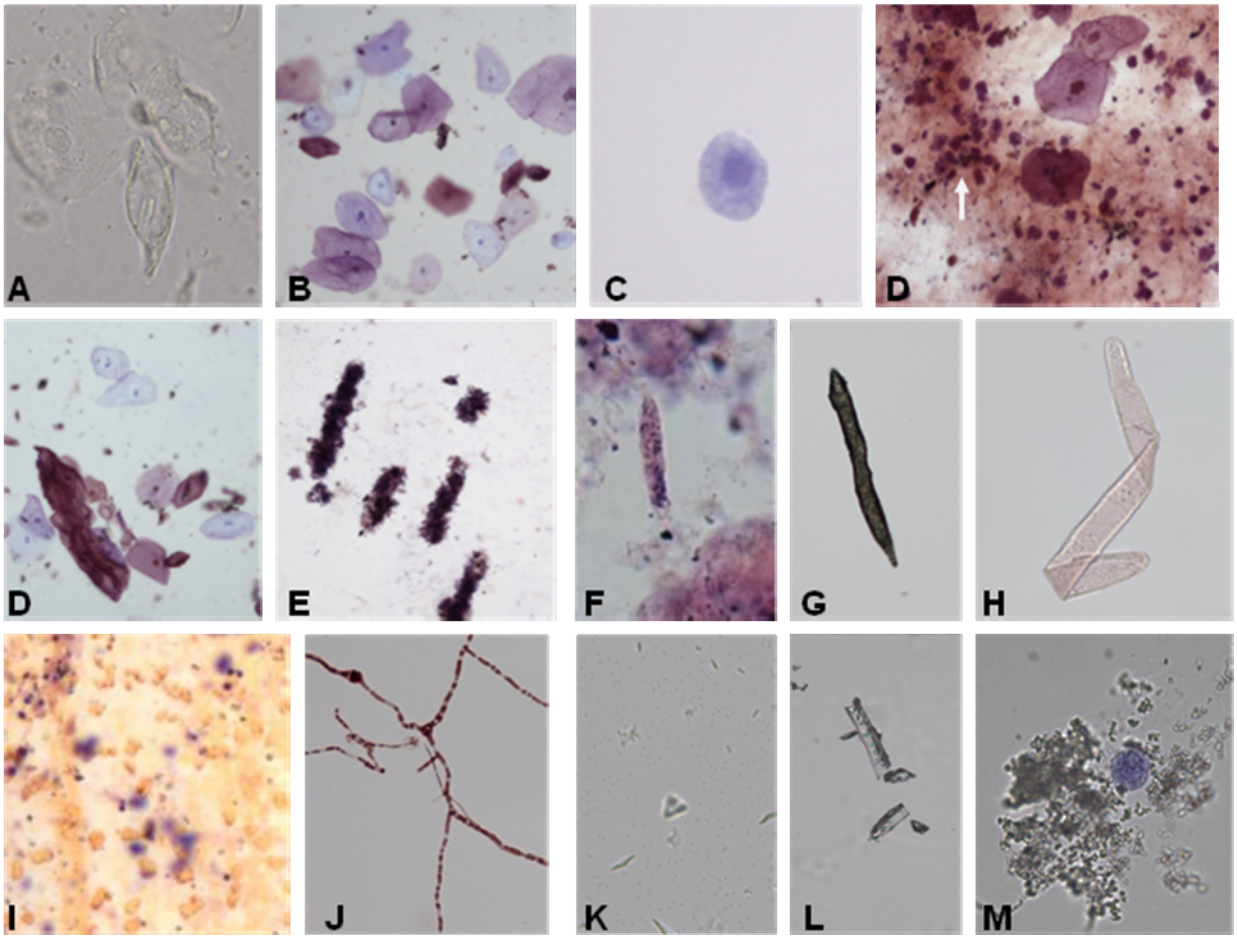

Figure 5.9 Common components of urine sediment Cellular components (top row) include squamous epithelial cells (A & B) derived from the urethra and transitional epithelial cells (C) from the bladder mucosae. Presence of red blood cells (D- arrow) in urine typically indicates contamination from the urethra or vagina. Casts (middle row) result from compaction of proteins or other materials within the renal tubule and collecting duct. Cellular casts (D) contain recognizable cells whereas granular casts (E-F) do not. Waxy casts (G) are believed to result from break down of granular casts. Lipid droplets (I) are visible as small yellow globules which float to the top of the coverslip and lie in a different focal plane than surrounding sediment. Presence of fungal hyphae (J) typically indicates post-collection growth of fungus in the sample or microscope slides (the latter is particularly common in humid areas). Urine crystals (K-M) are more common in acidic urine and can be identified by shape and refractive properties.

ACTIVITY 5

Effects of sodium, caffeine, and baking soda on urine pH and specific gravity

1) Each group member will consume one of the following within 5 minutes (each group member should choose a different item)

- 1 bottle distilled water (500 ml)
- Distilled water + Salted Pretzels
- Distilled water + 1 tsp Baking Soda
- 500 ml of Diet Soda

2) Obtain a urine specimen immediately after consuming the item in the previous step. Test this sample for pH and specific gravity and record this data as a "baseline".

3) Form a hypothesis as to what effects each treatment will have on: Urine Volume, pH, & specific gravity.

4) Thirty minutes later, attempt to obtain another urine specimen. Test your specimen as in #2, but also record volume.

5) Thirty minutes later (one hour after the start of the experiment) obtain a third sample. Measure volume, test for specific gravity and pH

6) Pool your own data with individuals from the other groups who consumed the same item as you. Include this table in your laboratory notebook.

7) Create a graph showing the effects of each treatment on the volume, pH, and specific gravity of urine samples for each collection period (Use average data rather than individual values)

8) Write a conclusion paragraph explaining your results.

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EXERCISE 12: Urinalysis

Table 5.1 Results of urinalysis on standardized urine sample

Patient Data							
Pet Name:				**Owner's Name:**			
Breed:							
Age:	**Sex:** M F			**Spay/Neutered?**	Y N		
Technician Name:							
Collection Date:	**Test Date:**			**Tester's Initials:**			
Physical Characteristics							
Color	colorless	yellow	amber	other	**Remarks:**		
	orange	green	red				
Appearance/Clarity	clear	hazy	cloudy	turbid			
Chemical Measurements							
Urobilogen (mg/dl)	Normal	2	4	8			
Glucose (mg/dl)	Neg	50	100	250	500	1000	
Ketone (mg/dl)	Neg	Trace/5	+/15	++/40	+++/80	++++/160	
Bilirubin	Neg		+	++	+++		
Protein (mg/dl)	Neg	Trace	+/30	++/100	+++/300	++++/2000	
Nitrite	Neg	Pos	(any pink color is considered positive)				
Leukocytes	Neg	Trace	+	++	+++		
Blood	Neg	Trace	Moderate	Trace	+/small	++/mod	+++/large
		Non-Hemolyzed		Hemolyzed			
pH							
	5	6	6.5	7	8	9	10
Specific Gravity							
	1.000	1.005	1.010	1.015	1.020	1.025	1.030	1.035	1.040
ERD							
	Negative	Low	Medium	High			
Microscopic Examination of Urine Sediment							
WBC_____/HPF	Crystals:			Parasites:			
RBC_____/HPF	Bacteria:			Spermatozoa:			
Casts_____/LPF	Yeast:			Artifacts:			
Epithelial Cells_____/HPF				Other:			
Comments:							

			Baseline			1/2 Hour			1 Hour			1 1/2 Hour		
Treatment	ID	Sex	Vol	SG	pH	Vol	SG	pH	Vol	SG	pH	Vol	SG	pH
Water Only														
pH:														
spg:														
Pretzels & Water														
pH:														
spg:														
Water & Baking Soda														
pH:														
spg:														
Diet Coke														
pH:														
spg:														

Urinalysis

Review Questions

1) Why are urine samples typically collected in from the first urination of the morning?

2) What sorts of activities/ metabolic states might lead to having a higher-than-normal blood or urine pH (alkalosis)?

3) What sorts of activities/ metabolic states might lead to acidic urine?

4) What's wrong with this picture?

5) What hormones are responsible for regulating urine volume?

6) Name three normal components of urine sediment.

7) Name three abnormal components urine sediment.

8) What condition(s) may be indicated by the presence of glucose in the urine? What other tests confirm you run to confirm this diagnosis?

The urine sample from a female patient tests positive for albumin. What diseases/conditions may be indicated? What other tests could you run to confirm your diagnosis?

[Type text]