

LABORATORY EXERCISE 7

PHYLUM CHORDATA

GENUS RANA –*Rana pipiens*

The frog, *Rana pipiens*, has been studied as the "typical vertebrate" throughout most of the history of biology, particularly in physiology. The study of muscle physiology began when Galvani observed that electrical stimulation of the frog leg, of its large muscles, or of the nerves to these muscles, will cause contraction. The "muscle twitch" is still studied on the leg muscles of the frog, and much of our knowledge of nerve-muscle physiology comes from studies of the frog. Heart physiology, heart contraction, the effects of various chemicals or nerves on the heartbeat, were all studied first in the frog. The advantages of the frog as an experimental animal are many: the whole animal is amazingly viable; the separate organs retain their living activities even after removal from the body, providing they are bathed with properly constituted physiological saline (Ringer's solution) and supplied with oxygen. The frog is a "cold-blooded" or ectothermic (poikilothermic) animal whose metabolic activities continue to occur in a wide range of environmental temperatures. Frog eggs, embryos, and larvae have been the subjects of many experimental studies of embryology and metamorphosis because they too can survive treatments that kill most other vertebrates. It is fitting that we dedicate at least one laboratory period to a thorough study of this invaluable animal. The observations you make on the living frog, and the techniques used to gain this knowledge, will be of use to you in any further work you do in biology.

BEHAVIOR. Observe the behavior of several frogs. Notice the normal stance as well as the position of the legs at rest and in jumping. Examine mounted skeletons of the frog to see how the legs are articulated to the pelvic girdle. How are the hind legs and pelvic girdle specially adapted for jumping? Touch the hind leg of a resting frog and watch its reaction (but also watch that the frog does not escape — anyone who lets a frog escape must catch it!). Note the method of breathing — does the chest or throat move? What is the sequence of the closure of the nostril valves? Touch the eye lightly and note the reflex reaction. Turn the frog over on its back and see how it rights itself.

PITHING. Frogs must be anesthetized before surgery in order to prevent pain to the animal and to relax its muscles. When it is not planned to have the animal survive, this can be achieved by removing the brain or destroying it by pithing. The instructor will demonstrate pithing, and will pith your frog for you if you ask.

STRUCTURES OF EXTERNAL ANATOMY. Examine the circulation of the blood through the capillaries in the thin webs between the toes of the hind foot. Spread the foot over the hole in the microscope stage, moisten the foot with saline, and focus on the capillaries under low power. How does the diameter of the capillaries compare with the diameter of the blood cells? Do the capillaries branch and join? Can you detect the heartbeat by the movement of the blood cells? Scratch the foot lightly with a needle — does this affect the blood flow?

Examine the skin for smoothness, moisture, and thinness. Amphibian skin serves as part of the respiratory system, absorbing oxygen from the air through the thin mucus secreted by the skin glands. Note the pigment splotches on the back.

Examine the eyes. Are there eyelids? Does the eye still retract reflexly when touched? Observe the thin nictitating membrane in the medial corner of the eye. Many vertebrates have this transparent membrane that sweeps across the eye to moisten it without completely blocking vision.

HEART PHYSIOLOGY. Pin the frog to a dissecting board, ventral side up, with pins through the tip of the upper jaw and each of the feet. Lift the skin over the abdomen, and cut it to one side of the midline from the pelvis to the tip of the lower jaw. The fluid-filled space beneath the skin is a large lymph sac into which oxygen is absorbed through the skin. **Keep the frog skin moist with saline during the rest of your dissection.** Reflect the skin to either side and pin it.

Lift the ventral body wall in the pectoral region and with only the pointed tip of your scissors make a circular "window" in the pectoral muscles and sternum just large enough to reveal the heart. A pericardial sac encloses the heart and constitutes a subdivision of the body cavity or coelom. The sac may be carefully torn longitudinally with the point of your sharp probe. The heart consists of three parts: two wing-like atria on the anterior end of the heart that contract in unison and slightly before the contraction of the single, thick-walled ventricle. The heartbeat has two stages, the systole (contraction) and the diastole (dilation). Blood enters the atria dorsally through veins from the lungs, head, and posterior parts of the body. It passes from the atria into the ventricle and thence anteriorly through the ventral aorta which divides into three pairs of arteries servicing the head, lungs and the rest of the body.

You will now conduct a series of experiments concerning the control of the heartbeat of your frog. During this time, it is **essential that the heart be kept moist at all times!** Since the physiological state of your animal is probably changing during the course of the tests, it is important that you bathe the heart with physiological saline at room temperature (RT) after each treatment, wait a minute or so to allow the heart to recover, and then *take another* "pretreatment" reading before testing the effect of subsequent reagents!

Immediately upon exposing the heart, flood it with RT saline., allow several minutes for it to stabilize, and take a "pretreatment" reading. Flood the heart again with saline solution at RT and take a "posttreatment" count. Is there any change? Why might there be? Flush the heart again with RT saline, take a "pretreatment" count, apply 15 drops of iced saline to the surface of the heart and then take a "posttreatment" count. Flush the heart again with RT saline and wait 1-2 minutes for the heart to stabilize. Take a "pretreatment" count, apply 15 drops of warm saline to the surface of the heart and then take a "posttreatment" count. What effect does temperature have on heartbeat rate? How do you account for this?

Allow the heart to stabilize several minutes by flushing it completely with RT saline and then test effects of the following reagents which have been made up in physiological saline at RT.

1. adrenaline (aka epinephrine)
2. acetylcholine
3. eserine (aka physostigmine)
4. atropine
5. acetylcholine and eserine (5 drops of each)
6. acetylcholine and atropine (5 drops of each)
7. serotonin

Use the same procedure (summarized below) as you used for testing the different temperature saline but use 10 drops of each reagent rather than 15 drops (except when combining 2 reagents, use 5 drops of each).

Take a pretreatment count

Apply reagent

Wait 15 seconds

Take a posttreatment count

Rinse with a dropper full of RT saline

Wait 1-2 minutes

Enter your data on the master sheet which will be in lab. This sheet, or the information on this sheet, will be made available to the class. Use the Microsoft Excel (see separate instruction sheet on use of Excel for data analysis) to test the class' results for statistical significance and save the data for a formal laboratory report on comparative heart physiology which you will prepare and submit later this term.

Upon completion of the heartbeat experiments, remove the heart and place it in a vial with saline. Does it continue to beat? Where does the stimulus for beating of the frog heart originate? Take a drop of blood from the pericardial sac, place on a slide, cover slip it, and examine. How do the erythrocytes differ from yours? Can you locate a leukocyte? How does it move? Can you find a thrombocyte?

STRUCTURES OF INTERNAL ANATOMY. Now turn your attention to the anterior end of your animal. Note the external nares (nostrils) near the tip of the upper jaw. Open the mouth and find the internal nares, just medial to the upper jaw about 1/3 the distance from its tip to its hinge. The nasal cavity is quite short in amphibians, since they do not have the palate which extends the nasal cavities to the back of the mouth as in mammals. Feel the two sets of teeth, the maxillary teeth on the upper jaw and vomerine teeth on the roof of the mouth. Are there teeth on the lower jaw? How do the teeth differ from human teeth? Rinse out the mouth with saline if it is clogged with mucus. In the back of the mouth find the following openings: Eustachian tubes, these join the ear cavities with the mouth and open just medial to the angle of the jaws; pharynx, the large dorsal slit at the back of the mouth that opens into the esophagus; glottis, the narrow vertical slit in the floor of the mouth that opens into the trachea. Flip the tongue out. How is it attached? How would it be used? Note how tightly the lower jaw fits into a groove in the upper jaw when the mouth is closed. What advantage would this have? Figure out how the frog would carry out these two necessary processes: getting air without water into the lungs; getting food and water into the

esophagus but not into the lungs. Dust a few carmine particles on the roof of the mouth to demonstrate the "mucus track". What function does it perform?

Extend your "window" with longitudinal anterior and posterior cuts so that the remaining viscera may be studied. Transverse cuts at the levels of the limbs will facilitate spreading and pinning the body wall. Note that the inner surface of the body wall is covered by a smooth, glistening peritoneum. The outer surface of each organ is similarly covered by a thin transparent layer of peritoneum.

Find the lungs, located in the body cavity dorsolateral to the heart. Are they contracted or expanded? By inserting a pipette into the glottis in the floor of the mouth, you may be able to inflate the lungs through the trachea, the tube that runs from the glottis to its bifurcation into two bronchial tubes that enter each lung. Cut off a small piece of lung and examine it microscopically. Is it a simple sac, or is it subdivided? Make a note of the lung structure for comparison with mammalian lung later. The lung may contain platyhelminthian parasites.

Use the chart of frog anatomy and the diagrams in your book to identify the following organs. The liver, three lobes of glandular tissue that are colored dark red by their rich blood vessel network; the gallbladder, a greenish sac lying under the right lobe of the liver - stores bile secreted by the liver and transmits it through the bile duct to the small intestine; the stomach, lying under the left lobe of the liver - it may be expanded or contracted. A short, wide esophagus runs from the pharynx to the upper or cardiac end of the stomach. The caudal end of the stomach is closed by a tough circular muscle, the pyloric sphincter, which opens to permit passage of food from the stomach to the small intestine. Cut open the stomach and examine its contents and the nature of its inner lining. Note the mesentery (connective tissue) that supports the stomach in a sling of peritoneum. The pancreas lies within this mesentery, dorsal to the stomach. The coiled small intestine is suspended from the dorsal body wall by a fan-shaped mesentery. Note the many blood vessels in this mesentery. Some are arteries carrying blood from the dorsal aorta to the intestine and others are veins carrying blood from the intestine to the liver.

The small intestine ends caudally in the large intestine, which opens into the cloaca (Latin for sewer). Find the urinary bladder, a thin-walled sac lying ventral to the large intestine. Cut through the pelvic girdle ventral to the bladder and trace the cloacal opening to the outside, the anus.

The frog is frequently infected with many kinds of parasites. Cut open the esophagus, lungs, large intestine, and bladder then examine each for parasites. Flukes, nematodes, and tapeworms commonly infest these organs, while encysted parasites are frequently found on the liver, pericardium, and body wall.

Identify the sex of your frog (be certain you observe the anatomy of both sexes). If it is a male, the medial toe on the forelimb will show a thickened pad of tissue around its base. If it is a female, the abdominal cavity may be filled with the enlarged ovary full of ripening oocytes (eggs). If this is so, carefully cut out the ovary on one side and remove it. Under the dissecting microscope, open the sac-like ovary in a dish of saline. Observe how each

oocyte projects inward into the hollow ovary on a short stalk of follicular tissue. Can you identify the dark animal hemisphere, the creamy vegetal hemisphere of each oocyte? After identifying the reproductive organs in your frog and ask someone dissecting the opposite sex to demonstrate those reproductive organs to you. Cut out the liver, stomach, and small intestine by cutting through their mesenteries. Do not remove the large intestine or cloaca, and do not injure the organs lying on the dorsal body wall.

Frogs of both sexes have large yellow fat bodies on the dorsal wall, just anterior to the kidneys. These organs store fat during the summer "eating season", and then serve as internal nutritive sources during the winter. Immediately caudal to the fat bodies lie the gonads, testes or ovaries, each fastened to the body wall by a mesentery. In the male, minute tubules, vasa efferentia, pass through this mesentery, carrying sperm formed in the testes to the sperm ducts embedded in the kidney. Remove one testis to a slide, mash it in saline, and examine for sperm. It will be necessary to use high power and to cut the light down to a minimum, but you ought to be able to see motile sperm. In the female, thick coiled oviducts lie on the body wall. These extend from a funnel-shaped opening located anteriorly behind the liver, to the enlarged ovisac in the pelvis. The mature eggs break out of the surface of the ovary into the coelom and then are moved by cilia on the peritoneum toward and into the funnel of the oviduct. As they pass down the oviduct, a jelly coat is secreted by oviductal glands around each egg. The eggs are stored in the ovisac ("uterus") until mating occurs, at which time they pass out through the opening of the oviduct into the cloaca and then out the anus. In the male, sperm pass through the duct of the kidney, down the enlarged ureters into the sperm sac in the lower part of the ureter. They are released through the openings of the ureters into the cloaca, and are shed over the eggs released by the female.

The kidneys are elongate, flat, dark organs lying tight against the dorsal body wall outside of the peritoneum. On the ventral surface of each kidney, identify the pale yellow streak of adrenal gland tissue. The kidneys are drained at their caudal poles by ureters that open into the cloaca. Between the two kidneys notice the large blood vessel, the posterior vena cava, the major vein that returns the blood from the caudal half of the body to the heart.

Remove the eye from your frog, and float it in a dish of water. Identify the cornea, iris, sclera and optic nerve. Cut it open around the equator, half way between the cornea and the optic nerve. Identify and understand the function of each of the following parts: cornea, anterior chamber, iris, lens, suspensory ligament, vitreous body, retina, fovea, blind spot, pigment layer, optic nerve.

Skin out a hind leg and take a small piece of pigmented skin then examine it with a compound microscope. Can you see the separate skin cells? What is their shape? Note the pigment cells and glands.

The muscles of the lower leg are an instructive illustration of vertebrate muscle-skeleton relationships. On the lateral surface locate the peroneus anteriorly, and behind it the large gastrocnemius. On the medial surface in antero-posterior succession locate the tibialis anticus, the extensor crurus brevis, and the tibialis posticus. By careful dissection locate or

determine for each muscle the origin(s), the insertion(s) and action(s). Which group of muscles act antagonistically?

At the end of the period the frog may be labeled and placed in an appropriate fixative for further study, or if you choose, wrapped in paper and disposed of properly.