

LABORATORY EXERCISE 5

PHYLUM MOLLUSCA

Molluscs are a very successful group of animals considering the number and variety of species distributed throughout all parts of the world. Members of the different molluscan classes show great variation in structure and behavior. For this reason, no single species can be truly representative of the phylum, and our choice of the living freshwater clams for this exercise is dictated primarily by their availability.

GENUS UNIO –*Unio sp.*

STRUCTURE OF SHELL. Examine the empty shell of a clam. The paired shells or valves are joined by a tough ligament which holds the interdigitating ridges of the two sides together at the hinge. Each shell increases in size by the addition of concentric bands of material secreted by the growing animal, and it is sometimes possible to tell the age of the animal by counting the bands on the shell. The shell consists of an outer horny layer, a middle layer of prismatic crystals of CaCO_3 , and an inner layer of iridescent mother-of-pearl. Are the skeletal elements living? The inner surface is smooth except for two large scars indicating the attachment sites of the muscles that close the shell and extend or retract the foot. Identify the dorsal (hinge) side, the anterior (short rounded) and posterior (long tapering) ends, right and left shells.

HEART PHYSIOLOGY: Compare your live clam with the empty shell, and locate on your clam the probable points of attachment of the anterior and posterior adductor muscles. Pry open the shells with a strong knife or probe, and insert a second sharp blade carefully between one shell and the soft mantle layer immediately internal to it. Work the point of the blade up to the level of attachment of the anterior adductor muscle, and scrape back and forth against the shell to cut the muscle attachment. Do the same for the posterior muscle and **slowly** lift the shell off, carefully freeing the underlying soft tissues from adhesions to the shell.

The body of the animal is covered by two halves of the tent-like mantle that secretes the shell on the outside and mucus on the inside. Edges of the mantle contain minute sensory receptors, particularly at the posterior end of the animal where the two mantles are thickened and held together to form two pigmented channels or siphons where the water enters and leaves the mantle cavity.

Cover the clam completely with conditioned water and add a drop of carmine to the water just beyond the siphons. Identify the incurrent siphon and the excurrent siphon. The shock of your dissection may have disrupted the flow of water through the mantle cavity, but if you allow the animal to lie undisturbed for a few minutes it should be restored. Look for the heart in the mid-dorsal line, just anterior to the posterior adductor muscle, and inside a thin-walled pericardial sac (the only true coelom of the mollusc). Cut open the lateral wall of the pericardium so that you can see the beating heart more closely. The heart is a shield-shaped bag, wrapped around the digestive tract which passes through the pericardial cavity. It has four angles, the anterior and

posterior extensions where blood vessels leave the heart, and the two lateral wing-like auricles where blood enters the heart through open holes. Does the clam have an open or closed circulatory system?

HEART PHYSIOLOGY. Remove the clam from the conditioned water and place it on a towel so that the heart is exposed and so the remaining valve has sufficient water in it to keep the organs moist. Count the number of "pretreatment" heartbeats in one minute and record them. Carefully apply 5 drops of the physiological saline on the heart, wait 30 seconds and count the number of "posttreatment" beats in a minute and record them. Is the "posttreatment" rate different from the "pretreatment" rate? If so, is the difference of physiological significance? Can you tell from your data alone? Why? Immerse the clam in conditioned water again for a few minutes (3-5 minutes) to rinse it. Remove the clam from the water and, following the procedure above, take a "pretreatment" reading, apply 5 drops of acetylcholine made up in saline, wait 30 seconds and count the number of "posttreatment" beats in a minute and record them. Why is it necessary to take a "pretreatment" reading again? After recording the acetylcholine "posttreatment" rate, rinse the clam in the same way as before and then test the effects of a serotonin/saline solution immediately following your recording of another "pretreatment" rate. Enter your data on the class master sheet. How do your data compare with those from other animals? Why are the data so varied? How might one "normalize" the data so that results from one animal can be compared with those of others? Are the results produced by all the animals more "significant" than the results of experiments with a single animal? Why? Would simple arithmetic mean values of all corresponding "pretreatment" and "posttreatment" values for all the animals give you better data? What is the likelihood that the "posttreatment" results you obtained on your animal are not due to the treatment but to some other phenomenon not related to the treatment? Can you place more confidence in the collective results produced on all the animals than on those you personally recorded? Why? Pinch the foot of the clam to see whether this has an effect on the heartbeat rate. How do you interpret the responses of the heart to all these stimuli in the light of what you know about synaptic transmission? At the end of this exercise after completing all of the other observations, remove the heart with a clean scissors, and place it in a small vial of physiological saline. Does it still beat? Why? Where does the stimulus for contraction originate? How is the beat rate altered normally? 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. Before next week, 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.

STRUCTURES OF INTERNAL ANATOMY. Deflect one mantle away along its dorsal attachments to the body, and identify the double gill plates, the muscular foot, the labial palps at the anterior end of visceral mass. Identify the mouth, a hole in the anterior end of the digestive tract, and two adjacent pennant-shaped labial palps, ciliated folds of skin that sweep water and food particles into the mouth hole. Remove one palp and examine it under the microscope. Drop very fine carmine particles on the palp to see whether or not the cilia move them in a particular direction. How does this relate to their method of feeding? Drop some carmine particles on the posterior end of the fill plate to check this process. Do different sized particles behave in the same manner? Why?

Cut off a piece of gill plate, being sure you know the dorsal-ventral, posterior-anterior orientation of the piece. Examine it on a slide with a compound microscope. This is the most favorable biological material for studying ciliary beat, so take time to observe here how cilia work. Is their motion coordinated? What is its direction with reference to the original position of the place in the animal? Remember, the compound microscope image has reversed and inverted the orientation of the specimen! Can the ciliary beat change? Test an anesthetic (0.5% chloroform) on the cilia. The gill plate consists of alternate stiff bars and water channels into which holes (ostia) admit the water flowing over the gills. Where are the respiratory organs of the clam? How does respiration occur? Are there any convective mechanisms for moving the respiratory medium? How do you account for the difference in mechanisms between this animal and other molluscs?

Eggs of the fresh-water clam are released into the water channels above the gills, where they are fertilized by sperm swept through the gills in the circulating water. The eggs develop within the gill channels into a hooked larval form, the glochidium. If either of the gills in your clam is thick and bulky, this may indicate the presence of developing eggs. The glochidia develop in the parent clam during the winter and are released in the spring through the excurrent siphon. They attach to the skin of fish or amphibians, where they live for a few weeks as temporary parasites until their development is completed. Has the parasitic mode of life resulted in any evolutionary change of the adult? Of the larva?

The behavior of the adult clam consists of limited movement and of periodically opening of its shell, sweeping water into the mantle cavity, and then closing the shell. The shell is held closed by tonic contraction of adductor muscles. The only known sensory receptors consist of chemoreceptors in the edge of the mantle near the siphons, and a primitive statocyst in the foot. The nervous system is equally simple, consisting of small ganglia near each of the adductor muscles and one in the foot, each sending nerve fibers to the adjacent muscles. How can you explain the great success, both in terms of survival of numerous individuals and long evolutionary survival, of an animal with such limited neurosensory equipment? Is this lack of cephalization a primitive or specialized characteristic? What is the evidence for your point of view?