LABORATORY EXERCISE 4

PHYLUM PLATYHELMINTHES

GENUS PLANARIA –*Planaria sp.*

With a soft brush, place a live *Planaria* in a small dish with a few millimeters of pond water. BEHAVIOR. Examine the behavior of the animal with a dissecting microscope. Study its movements in a thin layer of water, and then in deep water. Place an obstacle in its path. Turn the animal over with a brush and observe how it rights itself. Squirt a stream of water on its head, the middle part, and the tail. Are the reactions different? Drop a very small grain of salt on the head, then (after recovery) on the tail. Are the two ends equally sensitive? Place an animal in a few mm of water in the petri dish that has been painted half black, cover it and leave it for 20-30 minutes in a dimly lighted area. The animal's activity in the dish can be traced by removing it from the painted dish, pouring off the water, shaking out excess water, allowing the dish to air dry, and then dusting a light layer of talcum in the dish. The talcum will adhere to the mucus track of the animal, leaving a record of its activity.

FEEDING. Introduce one or two washed *Artemia* into a watch glass containing a planarian and make observations of the feeding behavior. Can a slow moving flatworm capture fast prey as *Artemia*? *Daphnia*? What is the function of the pharynx in feeding? You may wish to feed a planarian small pieces of fresh beef liver to check on the function of the pharynx. Try offering a larger live animal such as *Daphnia*. Does the planarian act differently?

STRUCTURES. Examine the prepared slides of *Planaria* or *Dugesia*. Identify the gastrovascular cavity, the pharyngeal chamber, the mouth, the branches or trunks of the digestive system, and the epidermis. Can you identify the cilia? What are the rhabdites?

OPERATIONS. After the worm is well fed, transfer it with a brush to the surface of a clean cork. Add enough water to keep the worm moist and flattened out. Cut the animal with a clean razor blade in the way you have planned for studying its regeneration. After making a careful record of the size and shape of each piece and its original position in the parent worm, transfer each piece to a separate vial half full of pond water. Plug the vial loosely with a small cotton plug, mark the vial so you can identify which piece it contains, and store upright in the area indicated. Examine each piece every day by removing it to a Syracuse dish. If the water in the vial becomes turbid from bacterial growth or degeneration of the worm, transfer the worm to clean vial and pond water. It is best to replace the water completely after each day's observation. At each examination, look for the colorless blastema, a rapidly growing mass of dedifferentiating-redifferentiating cells formed at the wound surface. Record when and where the blastema forms, when it becomes pigmented, and when it starts to differentiate into formed structures. Compare the rate and nature of the regenerative process of each piece with reference to its initial position and polarity in the parent worm. Is there evidence of a physiological gradient? A morphological gradient? Is there any relation between the size of the piece and the rate of regeneration? Do regenerated structures form only in the blastema, or is there reorganization of old tissues into new ones? How closely does the structure of the new worm resemble that of its parents; of its surgical siblings?

PHYLUM ANNELIDA

GENUS LUMBRICUS *–Lumbricus sp.*

Become familiar with the internal anatomy of the earthworm, *Lumbricus*, from the text and laboratory charts before starting your study. The purpose of your study should be to become thoroughly familiar with the behavior, structure, and tissues of the 'typical animal'.

BEHAVIOR**.** Place a live worm in a clean pan with a moist paper towel in the bottom of one half of the pan. How does the worm crawl when on the moist towel? On the smooth surface of the pan? Tilt the pan to test its ability to climb up a grade. Use your finger as a barrier in its path. Can you feel the bristles by which it moves? Test its reaction to light touch on the tip of the head, in the midbody, and near the tail. Is there any difference in the degree of sensitivity of the various regions? Observe carefully the sequence of contractions in the successive body segments. This is a clear example of behavior based on segmental reflexes: when one segment contracts, it stretches the muscles of the next segment, stimulating their sensory endings and starting a new reflex arc through the ventral nerve cord. When you touch one segment, do the more anterior segments contract, or the posterior segments, or both? Is there any difference in the speed of muscular contraction after a light touch as compared with a heavier stimulus? Turn the worm over. How does it right itself? Note that this is a much more complicated reaction than the similar behavior shown by the flatworm. The earthworm has two sets of muscles, a circular layer and a longitudinal layer. It has a bilaterally symmetrical nervous system. It is made up of sensory neurons whose dendrites go to receptors in the skin and muscles of the same segment, and motor neurons whose axons go to the muscles of the same and opposite sides of the body in the same segment or adjacent segments. Its basic behavioral reaction, that is peristaltic contraction of the muscles of successive segments, is modified by 1) differences in reaction on the two sides of the body, 2) crossing of nerve fibers from one side to the other, and 3) high speed nerve transmission from one end of the body to the other along specialized giant “through" fibers. Examine the behavior of the worm in light of these various pathways through its central nervous system.

EXTERNAL ANATOMY. (some of these structures may be more easily seen in an anesthetized worm under a dissecting microscope) Identify the <u>mouth</u>, the <u>prostomium</u>, and the <u>clitellum</u>, a thickened girdle of mucus-secreting skin in segments 32-37. On the ventral side, the <u>openings of the sperm ducts</u> are usually conspicuous on the 15th segment. The <u>openings of the oviducts</u> on segment 14, and <u>openings of the seminal receptacles</u> on segment 9-10 may be difficult to see**.** <u>Dorsal pores</u> located in the dorsal midline provide outlets from the coelom to the outside of the body. Immediately under the skin on the dorsal side is the longitudinal <u>dorsal blood vessel</u> which, by rhythmic contraction, forces the blood through the circulatory system. Examine the worm under the dissecting microscope to see the “beating" of this dorsal vessel.

Anesthetize the worm by placing it in a small dish of pond water to which you have added 5-10 drops of chloroform. Observe the worm's behavior during anesthetization. Body contractions usually squeeze yellowish coelomic fluid out through pores located in the dorsal body wall between segments. When motion ceases, test the worm for reactivity to touch on the head. When it no longer reacts, cut off the posterior third of the animal with a sharp razor blade. Remove the posterior third to fresh physiological saline (0.9% saline). Cut open this posterior piece along the dorsal mid-line. Examine the body cavity near the tail for brown encysted masses of parasites. Remove one, smash it under a coverslip on a slide, and observe it with a compound microscope. To what phylum do the parasites belong?

INTERNAL ANATOMY. Pin the prostomium to the side of a dissection board with the darker dorsal side up. Cover the worm with chloroform solution. With a scissors, cut through the skin just to one side of the dorsal blood vessel. Keep the points of the scissors parallel with the skin so that you do not puncture the internal organs. Lift each flap of skin with a forceps and with a blade or needle cut the septa that divide the internal body cavity into segments. Pin each half of the skin open on the dissecting board, like the pages of an opened book. The worm should be pinned parallel and 2-3 cm from one edge of the dissecting board so that it can be placed under your dissecting microscope for later observations. Make sure to identify the following structures:

<u>Pharynx:</u> The bulbous muscular anterior part of the digestive tract. Note the many radiating muscles that attach it to the skin and aid in sucking in soil as the worm eats its way through the earth.

<u>Esophagus;</u> A slender yellow-brown tube running the length of the body from the gizzard to the anus. The yellowish color is due to special pigmented cells, <u>chlorogogue</u> cells, that cover the outside of the intestine. These cells store glycogen when the worm is well fed and release it during starvation; they may also play a role in excreting waste chemicals from the gut into the coelomic fluid. Examine a prepared microscope slide of a transverse section through a worm, and identify the body wall, coelom, and digestive tract. Note that the lining of the gut is folded into the cavity in a longitudinal hanging partition, the <u>typhlosole</u>. The cells covering the typhlosole as well as those lining the gut absorb the chemicals released by digestion in the crop and gizzard. Examine the chlorogogue cells covering the outside of the gut and note the large amount of cytoplasm each cell has. Identify the other organs and tissues of the earthworm shown in the slide.

<u>Dorsal hearts:</u> 5 pairs of thickened block vessels running around the esophagus from the dorsal blood vessel to the ventral vessel. Are they still beating? They may be hidden by the septa against which they lie, but can be seen with the dissecting microscope if the septa are turned back. Cut out one of the hearts and examine it on a slide with a compound microscope. Does it continue to contract? Examine the blood that oozes out of the heart. The worm does not have red blood cells, since the hemoglobin is in solution in the plasma, but it does have cells similar in function to our white blood cells.

<u>Seminal vesicles;</u> Three pair of large white sacs containing sperm released from the testes that lie inside the vesicles. Remove one seminal vesicle to a slide, crush it in a drop of saline, cover and examine for <u>sperm</u>. Note the various stages in maturation of the <u>sperm</u>. Usually the sporozoan protozoan, *Monocystis*, parasitize the seminal vesicles, invading the sperm cells and living off their protoplasm. Carefully cut off the dorsal wall of the anterior pair of seminal vesicles, flush out the sperm, and examine the ventral body wall

to see the testes, a pair of round white bodies lying on the posterior faces of septa 10 and 11. The sperm released from the testes are collected into the funnels of the sperm ducts that run from the seminal vesicles to a pair of openings on the ventral body wall in segment 15.

Sperm receptacles; Two pair of small, white, round sacs attached to the ventral body wall in segments 9 and 10, just anterior to the seminal vesicles. These four blind sacs open only to the outside; they receive and store the sperm of another animal in copulation. Remove one receptacle to a slide to see whether or not it contains sperm.

Ovaries; Two small, triangular, white organs attached to the septum of segment 15. Each ovary contains eggs in various stages of maturation. Mature eggs drop off the ends into the funnels of the oviducts, lying on the ventral body wall, opening to the outside on segment 14. These are difficult to find in a fresh specimen.

During copulation two worms cling together by secreting a mucus sheath around themselves, with the head of one worm caudal to the clitellum of the other worm, heading in opposite directions. Sperm are exchanged from the ducts of each worm into the sperm receptacles of the other worm. The two worms separate, and later each secretes a mucoid capsule from its clitellum. The capsule then slides anteriorly over the body, like a girdle. As, it passes segment 14, eggs are released from the oviducts, and as it passes segment 10-11 the foreign sperm are released from the sperm receptacles. The capsule slips off the head of the worm, and its ends contract to make a cocoon enclosing the fertilized eggs. The young worms develop inside the cocoon.

Nephridia; A pair of transparent coiled tubules located on the ventral wall of each segment. Examine the ventral body wall with a dissection microscope and locate the nephridia. Each nephridium consists of a ciliated funnel that opens out of the coelom of one segment, a coiled duct that passes through the septum into the next segment and opens out of the body through a ventral nephridiopore. The tubule is surrounded by a capillary network, branching off of the ventral blood vessel. Coelomic fluid is swept into the open funnel by ciliary currents, passes through the coiled tubule where some of the chemicals are resorbed into the blood, and the remaining fluid is excreted through the nephridiopore. By careful dissection remove several nephridia from their attachment to the body wall, cut the septum just anterior to each nephridium, and remove both together to a slide. Add saline and a few grains of carmine powder, cover and study under high power. Light pressure on the coverslip should spread the nephridia out flat so that you can examine all parts of the tubule. Identify the funnel shaped nephrostome, and observe the movement of carmine grains down the tubule. Nematode parasites frequently live in the nephridia, so do not confuse their thrashing with the ciliary beat. Do cilia line the entire nephridial tube? Do the carmine grains move slowly or rapidly through the tubule? The Annelid nephridium is of special interest to zoologists because vertebrate embryos develop a similar excretory tubule as part of their first kidney, the pronephros.

Nervous system: A 'brain' or pair of ganglia lies in the third segment of the head. Use a dissecting microscope, push aside the muscles that attach to the pharynx, and locate the two supra-esophageal ganglia. Nerves enter the ganglia from all parts of the skin of the prostomium. Two large nerves leave the ventral side of the ganglia, pass around the esophagus to two similar sub-esophageal ganglia. The ventral nerve cord starts from these two ganglia, and runs posteriorly, the length of the body, under the intestine. Remove the intestinal tract and examine the ventral body wall under the dissecting scope. Identify the large double ganglion and the three pairs of nerves that run to and from the body wall in each segment. These nerves are formed by both sensory fibers entering the ganglia and motor nerves running from the cells of the ganglion out to the muscles of the body wall and the gut wall. Three special 'giant fibers' run the length of the body through the nerve cord. These are coordinating nerve fibers; large in size and therefore capable of rapid conduction; interrupted in each segment by special types of synapses through which rapid transmission occurs.

Most of the behavior of the earthworm is controlled by segmental reflexes, and small isolated segments of the worm will continue to contract until the tissues die. But some of the total reactions of the worm are controlled 1) by the supra-esophageal ganglia, which receive sensory fibers from the prostomium and give rise to inhibitory fibers and 2) by the sub-esophageal ganglia, which give rise to the giant fibers. If the supra-esophageal ganglia are removed, the worm loses sensitivity on the head, but its reactions to stimulation on other regions becomes excessive, even convulsive. If the sub-esophageal ganglia are removed, the worm becomes sluggish and its reactions uncoordinated. Locomotion is controlled largely by segmental reflexes.