

BIOLOGY 340
Comparative Embryology

Laboratory Exercise 1
Laboratory Examination of a Basal Deuterostome:
Sea Urchin Embryos

Introduction

Many laboratories that examine sea urchin embryos do so with live animals. This strategy however, is affected by seasons, animal availability, and the frequent desiccation of embryos under the hot lights of microscopes. Thus this exercise is done with preserved materials. This limits the number of diversity of embryos that may be observed and often does not allow for viewing of more advanced embryonic stages; however, it is more reliable in other regards.

Before starting this laboratory, be sure to review with your laboratory partners (and the laboratory instructor if necessary) proper care and use of compound microscopes. As always, be sure to keep scopes and objectives clean, and never, never, ***never*** focus down into a slide. Much pain and suffering awaits the student from whose station the instructor hears the telltale “crack” of a slide.

Materials

A small box containing up to a dozen prepared sea urchin embryo slides will be available in the laboratory. Each group of students should take one of these along with a microscope for use with the slide.

Each slide preserves hundreds of sea urchin (*Arbacia* sp.) embryos of all different stages. Most common are 2-cell to mid-gastrula stages, though a few single-celled zygotes as well as prism and pluteus larval stages can be found. The latter will require quite a bit of hunting.

Hints

The common tendency is to crank the illumination way up and open the microscope’s diaphragm entirely. Remember that these are very small organisms, easily washed out of view from too much illumination. Start on low to medium levels of illumination to find the embryos. Additionally, this will give a slightly greater depth of field for viewing.

Recall also that the preserved organisms you’re looking for are three-dimensional organism. Thus, you will not always see everything in an organism in one focal plane. You will have to focus up and down with the fine focus to see all structures. This introduces you to one of the

great exercises of comparative biology – the understanding of three-dimensional organisms from a series of two-dimensional images.

Images on pages 72-73 of your laboratory manual will be the most similar to the views you will have.

Examination of Sea Urchin Embryos

Note that the preserved slides include a mix of all different stages illustrated in your laboratory manual. You will have to scan around the area of the slide, as well as up and down through differing focal planes to see all that your slide has to offer. Do not hesitate to view those of your fellow students if they find a particularly well-defined example.

2-, 4-, and 8-cell Stage Embryos

See figures 1.1 to 1.4 in your laboratory manual for examples. Embryos of this stage are still essentially solid spheres of cells. As such, they will have the same mass as blastula and gastrula stage embryos, but will be of slightly lesser diameters. Note that as three-dimensional structures, they will require some fine-focusing to see the entire embryo, and especially, to see cleavage planes between cells. Be sure to note the radially cleaved arrangement of the individual cells.

Exercise: In the space provided below, draw representative examples of embryos of the 8-cell stage or less.

16- to 32-cell Stage Embryos

See figure 6.15L-M in your laboratory manual for examples. By this stage, animal and vegetal poles should be distinct. Cells of the vegetal pole will appear slightly larger in lateral view. However, if you can find an embryo that is visible from the direction of the **vegetal pole**, be sure to try to identify the **micromeres** (that will eventually give rise to primary mesenchymal cells).

Exercise: In the space provided below, draw representative examples of embryos of the 16- to 32-cell stage embryos.

Blastula Stage Embryos

See figures 6.14-I and 6.15M-P in your laboratory manual for examples. Numerous embryos of the blastula stage should be visible in your slides. Some of them will be early blastula stages that appear as simple hollow spheres. Find examples, noting the epithelial surface boundary of the **blastula** as well as the **blastocoel**. Some will be slightly more advanced. With focusing through a range of focal planes, you can often spot **primary mesenchyme** cells (e.g. figure 7.3A in your lab manual) beginning to ingress into the blastocoel. In some cases, the primary mesenchyme cells will simply appear as cells beginning to detach from the vegetal pole and plate. In other cases, they will begin to assume a columnar orientation relative to the vegetal plate.

Exercise: In the space provided below, draw representative examples of blastula stage embryos.

The Gastrula

See figures 7.3A-G in your laboratory manual for examples. The **gastrula** is the stage when the gut tube begins to form. A variety of early to later gastrula stages should be observable in your slides. The earliest stages of **gastrulation** can be seen in embryos that show a shallow dimple – the beginning development of the **blastopore**. At least one or more of these is usually found in the slides and you should try to do so.

Focusing through the different focal planes through the embryos, look for embryos that demonstrate the extension of the primitive gut, or **archenteron**. If it is only reaching approximately half way through the embryo or less (as in Figure 7.3B-F) you are seeing the invagination of the primary endoderm. (Note, you will not always find a gastrula in the conveniently lateral view. You might encounter one or more where you are looking directly down the long axis of the gut as in Figure 7.3D.)

Search for progressively more advanced stage gastrulae, seeking out the extension of the archenteron all the way to the opposite epithelial wall of the embryo (as in Figures 7.3E-F).

Exercise: In the space provided below, draw representative examples of gastrula stage embryos.

Prism Larval Stage

See figures 7.3G-H, 7.4, and 7.5 in your laboratory manual for examples. Prism and pluteus larval stages are much more difficult to find on the slides provided. If you find one, the other, or both, alert the laboratory instructor as well as your fellow students!

If you find a prism larva, focus up and down with the fine focus and attempt to identify the apex of the animal where the apical tuft will be. On slightly later prism larvae you should be able to make out the beginnings of the **anal arms** and an **oral lobe**.

Exercise: In the space provided below, draw an example of a prism larval stage embryo.

Pluteus Larval Stage

See figures 7.4 and 7.5 in your laboratory manual for examples. As mentioned previously, prism and pluteus larval stages are very difficult to find on the slides provided. If you find one, the other, or both, alert the laboratory instructor as well as your fellow students!

If you find a pluteus larva, focus up and down with the fine focus and attempt to identify the apex of the animal. Attempt to distinguish the **oral arms** from the **apical arms**. Elongate supporting structures running the length of each side of the embryo are skeletal structures, the **spicules**.

Exercise: In the space provided below, draw an example of a pluteus larval stage embryo.