LABORATORY #8 CENTRIFUGATION AND COMPRESSION EXPERIMENTS ON FROG EGGS

MATERIALS: mature gravid female frogs, injected three days earlier with pituitaries; mature males frogs. Centrifuge tubes, Holtfreter solution, clinical centrifuges, slides, permaplast clay, culture dishes. Water used must be dechlorinated or 10% Holtfeter solution made in distilled water.

Artificial insemination is accomplished according to the directions from Ward Supply House. The eggs are observed during the washing with water after removal of the sperm, so that they are not disturbed until rotation is complete. However, the dish can be observed to watch polar body formation which shows up as a white spot in the middle of the black animal hemisphere. Grey crescent formation can also be observed during that time. After only black egg surfaces are uppermost, slide a coverslip under the eggs to separate them from the petri plate. Then cut the egg clusters into groups of 6-10.

CENTRIFUGATION. Place 12 eggs into a centrifuge tube of water. SPIN FOR SET TIMES, settings I, II, III for 1-5 min, RECORD THE TIME OF THE CENTRIFUGATION AND HOW LONG AFTER FERTILIZATION IT WAS DONE. If you want a more uniform effect, allow the eggs to rotate after placing them in the tube, so that all animal poles are upward. Observe the centrifuged eggs, which have layered cytoplasm, draw their appearance. Those done at speed 1 for short times will have little stratification (and little abnormal development.) Those done at speed III will mostly die before gastrulation. Those which have large redistribution of cytoplasm will also probably have abnormal development, though some will be normal. There is a remarkable difference in susceptability to treatment in a population of embryos. There is a bell-shaped curve so some are effected more or less than others. Why is that? Place treated embryos into culture dishes clearly marked with the time of centrifugation and time after fertilization. Place a cover on the dish, which has small air holes in the top. Culture them until the next lab period, observe and record the results. (the normals vs. the abnormals, and the types of abnormalities.) Do four times, or speeds and have a control dish which had the embryos taken into and out of the centrifuge tubes in the same way.

COMPRESSION EFFECT ON CLEAVAGE PLANE. Take groups of 6 eggs at about one hr post-fertilization and place in a compression slide made by putting narrow roles of clay between two glass slides, with the bottom one having the eggs on it. The eggs should each be in a droplet of water, but too much water makes the clay come unglued. Compress the slide until you can see a visible change in diameter. Place the slide the animal pole upward on the table and observe the cleavages. Draw the eggs at each cleavage. The times of cleavage are shown in the table at the and of the exercise. Observe normal cleavage in comtrols placed between two slides, but not

compressed. Note differences. Remove the eggs from compression at the 16 cell stage. Place into a culture dish. Observe at the next lab period to see if there is abnormal development.

ANSWER SHEET FOR LABORATORY #8

NAME_____

1. Describe the appearance of the centrifuged eggs immediately after it is done.

2. Describe the appearance of the embryos in later development.

3. How does compression change the cleavage planes?

4. Does alteration of cleavage planes lead to abnormal development?

5. What kinds of cell particulates are disarranged by centrifugation or inversion, and what kinds are not?

6. How could this cause abnormal development?

7. Were there any abnormal embryos in the controls? How do you explain that?