CHICK CELL DISSOCIATION AND REAGGREGATION

PURPOSE: To compare various early embryonic tissues in terms of how easily they can be dissociated, and what kinds of mechanisms are involved.
Materials: Chick Ringers solution, Hank's Balanced salt solution (a phosphate buffered saline with glucose and phenol red (a detector of pH)); scalpel, petri dishes, microscope, at least two early chick embryos.

Method: Embryos can be candled if they are over 2 days old. On the earlier ones, you just have to open them to see if they are alive. Either break them into chick ringers or break the shell back to expose embryo. When you are ready to remove the embryo, firmly clasp the edge of the embryo blastodisc with forceps in your left hand. Do not let go at any time until this operation is complete or you will lose the embryo into the yolk. Cut with scissors around the blastodisc using your right hand, including around the forceps so they are holding the embryo you are cutting off. After cutting completely around, remove the blastodisc by picking up the forceps and place it in a petri dish with ringers and gently flatten it out. You must keep the embryo warm with your light for the rest of the experiment, or place in the warm incubator between periods of observation. Now we will do the same so that we have two embryos of approximately the same stage.

The embryo will be placed in Ca-Mg-free Hank's solution and cut into pieces such as <u>brain, heart, somites, yolk sac,</u> and if any <u>amnion</u> is presnt, use that. Now cut each piece into 2. That means you will have a total of four pieces of tissue of each type from the two embryos. We will place one of each set of four in a control Hank's solution, and the other three in different experimental solutions used to dissociate cells.

The kinds of solutions used will be 1) Ca-Mg-free Hank's plus 0.05% trypsin, pH 7.2, 2) Ca-Mg-free Hank's plus 0.05% trypsin and 0.005M EDTA and 3) Ca-Mg-free Hank's plus 0.005 M EDTA. Now use vaseline to separate the petri dihes into four compartments; mark the dish and each well, after placing 1/2 ml of the different solutions on different pieces of the same tissue in the different quadrants of the petri dishes, record the time, cover, and place in the incubator at 37 degrees.

Decide how you are going to score your results, for example by the size of clumps under the microscope, number of cells per clump, effect of mechanical stirring with needle, effect of gentle pipetting with a pasteur pipette.

After 10 min observe each piece and record any change in appearance. Using a needle test the tissue for firmness, see if it is starting to fall apart. Replace in incubator and reobserve at 10 min intervals for 1 hour recording the exact time of observation. If nothing happens after 30 min, use some mechanical method to aid, for example stir with needle or gently pipette. Record the methods used. If a particular tissue falls apart much faster than the others, take note of it. <u>Record what kinds of adhesion mechanisms you think are</u>

being interfered with in each case, using your text book as a resource.

Experiment with dissociated cells: pipette some dissociated cells into a centrifuge tube, add ten times the volume of Ca-Mg-free Hanks and spin down gently, remove and toss fluid. Now add 1 ml Hank's with Calcium back and see if they adhere after 30 min in the incubator in the centrifuge tube or in a petri dish on the shaker at 37 degrees. If you have several kinds of tissue dissociated, mix some of them and see if they reassociate with Ca++.

RESULTS:

Name_____

Questions to answer: At what time did each tissue start to dissociate? Which tissue was the fastest to dissociate? Which solution did it the fastest? Which did it the most completely? Check the size of the clumps and number of cells per clump unde
r the microscope to answer that question. How would doing the experiment at room temperature effect the experiment? Did the heart continue to beat in the solutions used? When did it stop?