LABORATORY #9

TRANSPLANTATION AND EXPLANTATION AND REGENERATION EXPERIMENTS ON FROG EMBRYOS

MATERIALS: NEURULA STAGE (62 HRS AT 18'C), TAILBUD (72 HRS AT 18'C), BLASTULA (1 DAY 18'C), GASTRULA (1 DAY 25'C), TADPOLES (3 DAYS 25'C); HOLTFRETER'S SOLUTION; WAX DISHES; FINE GLASS NEEDLES, WATCHMAKER'S FORCEPS, BUNSON BURNERS AND NEEDLE PULLER FOR MAKING FINE NEEDLES.

Prelab preparation: Look up the effects of extirpation of various anlage at various stages; look up determination, induction, self-differentiation. Decide what areas you will try to transplant and the expected results of putting that area onto various stages of embryos, in various locations. See the potency sheet at the end of this experiment.

EXPLANTATION AND EXTIRPATION. THE HARDEST PART ABOUT SURGERY ON EMBRYOS IS THE REMOVAL OF THE FERTILIZATION MEMBRANES AND JELLY COATS.

Remove the jelly if *Rana pipiens* by rolling eggs on damp paper towels using the large forceps. Use the watchmaker forceps only on wax lined dishes. In Xenopus it has to be done by hand with fine forceps.

Place the dejellied eggs in 100% Holtfeter's solution, in a wax lined dish and remove the fertilization membrane using two pairs of watchmaker forceps. Try to catch the membrane with one pair and tear if off with another pair. Don't be too gentle at first, ruin some eggs until you get the feeling of how delicately you have to treat them. They have amazing healing powers.

EXTERPATION. If you tear some embryos up doing the removal, observe the cells in different regions of the embryo as far as pigmentation, size, and ability to heal over a wound. Once the membranes are removed, take two glass needles and use them like scissors across each other to cut a piece out of the embryo.

Transfer the embryo and explant into a culture dish with 10% Holtfreter's solution with antibiotics after the wounds have healed for at least an hour. Abnormal development will occur if you try to grow the embryo for too long in full strength Holtfreter's solution. Be careful to have the transfer pipette full of water and to immerse the pipette into the culture dish before expelling the contents. Otherwise, the embryo will get caught in the surface tension and be instantly homogenized. Make drawings of your successful operations.

It would be wise to do two different stages: blastula or gastrula and neurula or tailbud. Removal of dorsal lip, neural fold, optic vesicle, lens placode, visceral arch, heart field, kidney field, blastocoel roof have all been successfully tried in the past. Observe the results at the subsequent lab periods and record the data. Remove any disintegrating pieces of

embryo from the culture dishes.

TRANSPLANTATION. For transplantation, the same kind of explant is removed, but it is placed into a wound in another host embryo, so the donor can be an extirpation, and the host a transplant. In order for successful transplants to be done, the wound must not heal before placing in the transplant, so the surgeries have to be done rapidly. Therefore, remove the membranes from two embryos, and then put them close to each other and make the cuts in each embryo without removing the tissue. Then remove the piece of each and either place them both into the other embryo or allow one to be an explant. One possible explant is the insertion experiment of Mangold, where a piece of embryo is placed in the blastocoel of an early gastrula stage.

TADPOLE TAIL REGENERATION. Observe the tadpole stage. Look for circulation of red blood cells in the external gills and see if the operculum covering the gills grows at the same rate on both sides of the embryo. Look at the eye development, pigmentation, gut whorls, and compare to the experimentals. Use one dozen tadpoles of uniform size. Measure their length. You can anesthetise them by placing them in a petri dish on ice. Cut off their tails using a razor blade after transfering them to a piece of cork. Make cuts according to the illustration, making sure not to cut away anal tissue, or too close to the abdomen. Place them in Holtfreter's solution for an hour so they can heal, before placing them in 10% Holtfreter's solution for culture. Make drawings of the cuts and the results the next time.

ANSWER SHEET FOR LABORATORY #9
NAME_____

1. What is the difference in potency of the presumptive eye region in the late gastrula and the neurula?

2. How can a. Explantation or b. Transplantation tell us if a tissue is determined, with capacity for self-differentiation?

3. What is a blastema?

4. What stage were the embryos you did your surgeries on where you find organs missing after extirpation?

5. What is regulation in a field?

6. What is the difference between a field and a primordium?