

## EFFECT OF TEMPERATURE ON CHICK HEART RATE

**MATERIALS:** Live chick embryos of two or three different ages, 48-72hr, 4-6 days, 9-12 days. Finger bowls, chick saline, warming plate, thermometer, stop watch, chick incubator. Chick saline: .9% NaCl, .042% KCl, .024% CaCl<sub>2</sub> (that is .9 g, .042 g, and .024 g per 100 ml.)

**PRELAB:** Read about the changes in circulation, heart fusion from two heart fields, neural control of the heart beat. This lab will show that during development, the embryo has little control of its physiology and behaves like a cold-blooded animal. If the environment is cold, it will grow cold and die, if the environment is hot, it will get hot and die.

During early development the heart rate is controlled by the ventricle, then when the atria fuse, by the atrium; then when the sinus venosus fuses, it becomes the pace maker (at about 72 hr.)

### **METHOD:**

Gently open a chick egg into enough warm saline (less than 40 degrees centigrade) to cover the egg. If the blood vessels break, the circulation will stop after the shock of bleeding (they have some clotting ability, but not great). So we must be careful not to cut the blood vessels with the sharp shell. Therefore, I usually open the egg under the saline solution and gently let it fall out of the shell. Fingers are the best instruments for freeing any parts stuck to the shell or to turn the embryo upright.

The heart rate can be observed through a dissection microscope or can be counted without any magnification. On older embryos where the amnion covering makes it impossible to see the heart, count the pulse in a large extreembryonic artery in the allantois or yolk sac. Record the temperature where you start, and count the heartbeats for a full minute. Don't try to take shortcuts and multiply, and you will find out why during the data collection. Slowly change the temperature, you can just let the solution cool off to room temperature, then place the dish on some ice to cool down to about 20 degrees. Record two readings every few degrees. Then warm the embryo back up by adding hot saline, and removing cold saline, again recording the rate of heart beat on the way up every few degrees, taking two sets of readings for each. Have different people count the heart rate and get an average. Take the temperature up to 42 degrees. Graph your results for both the way down and the way back up.

What conclusions can you make about the relationship of temperature to heart rate? Did you get the same results for all the stages of embryos?

## EFFECTS OF CHEMICALS ON HEART RATE

If you have some chemicals try the following:

You want to add only one thing to each embryo, to check out the result on heartrate. Use the earliest stage of embryo and cut the whole embryo off the surface of the egg. To cut the embryo off the egg, insert your forceps just outside the blastodisc (outside the blood circle) and cut around the entire blastodisc and forceps with some scissors. Then transfer it using a spoon or spatula under it to a petri dish of saline with one of the ingredients below. Make sure they are all warm, at the same temperature. This requires quite careful students.

An alternative approach is to break the egg into a petri dish, cover it with plastic wrap so that it touches the embryo and place that on a constant temperature pad which has been heated to 50 degrees in an oven or boiling water bath. The pad becomes clear when the correct temperature, mix the contents of the pad before placing the petri dishes on it. Place a thermometer on the pad to be sure the temperature remains the same, since we only want to vary one independent variable at a time so that when we measure the dependent variable (the heart rate) we will know the cause of the change.

Measure the heart rate several times before adding the chemicals, for each chick. Then you can compare the rate after the addition and see the change if there is one.

Students can measure the effects due to different things which interest them. They should decide the day before so they can bring from home the things they want to test. (Coffee, tobacco extract, antihistamine, calcium tablets, vitamins, retina, alcohol (only a few drops are needed), bicarb. These can be added to the chick saline before adding a few drops under the plastic wrap. Or:

Add vinegar.

Use a saline made only with KCl- .6M KCl (4.5 g/100ml). This was the most effective agent I tried- it slows the heart almost to a stop. The effect can be reversed by adding many drops of regular saline.

Add some powdered coffee

Add some tea leaves.

Add a vital stain so the structures become more visible.

Insecticide.

For observation of chick embryos over several days, they can be grown in chambers made from plastic drain pipe as shown in the article: Effects of varying chamber construction and embryo pre-incubation age on survival and growth of chick embryos in shell-less culture. BE Dunn, TP Fitzharris, BD Barnett. Anatomical Record 199:33-43. 1981. If embryos become

broken or bleed during their opening, they can still be used to look at under the microscope to count the somites or to watch the heart beat in saline, look at differences between different ages. Just grab the embryo with your forceps and don't let go and get under it with a spoon, transfer to a petri dish or slide. These are good for doing experiments over several days.

Graphs can be made of the heart rate versus temperature or age.

#### DISCUSSIONS:

Effects of high  $K^+$  concentration: removal of membrane potential so that it cannot respond.

Effects of high  $Ca^{++}$ : discuss effect of  $Ca^{++}$  on actin and myosin interaction.

Many agents that effect the adult heart through the nerves do not effect the embryo heart because the nervous system has not yet developed connections to the heart, and the brain control mechanisms appear very late in development. That is why it is so important that the hen or the incubator maintains the proper temperature for the embryo.

## HOW TO BUILD A CHICK INCUBATOR FOR ABOUT \$15.00

(If you have \$50 available, you can get a small 50 egg incubator from the Sears Farm catalog. The same incubator sells for \$125 in scientific catalogs.)

SUPPLIES NEEDED: thermostat for 100 F\*, heater coil (Eagle straight glo-coil)\* or light bulb, used computer fan with cord\*, porcelain bulb socket\* (all starred items were purchased for \$13.50 at Apex Electronics, a used and surplus store, 8909 San Fernando Rd, Sun Valley, CA. 818-767-7202), three screw wire connectors, cord with plug, xerox paper box with lid (or styro-foam cooler), thermometer, dish to hold water, small sponge for dish (keeps water from spilling), scissors to make holes in box.

Make holes in the lid the size of the thermostat and the porcelain socket and push them through to the inside of the box, just leaving the wiring and the end sticking outside. Place the fan at one end (not touching the end) of the box and make a hole the size of the cord so it can be stuck through to the outside.

Wiring: take one wire from each of the thermostat, heater socket, and fan and screw them together. Take one wire from the thermostat and screw it together with one wire from the plug-in cord. Hook the other wire of the plug-cord to the remaining wires from both the fan and the heater. Now the fan and heater will only come on after the incubator is plugged in when the temperature is lower than the thermostat is set. To set the thermostat, make holes in the lid the size of your thermometer and then five other holes (I used my 3/8 inch drill bit). Make two holes in each side of the box, and one in each end. These holes are air holes. Now place your dish of water with the sponge in it in the box and leave room for a wire basket of eggs. Replace the lid and plug in the cord. Make sure the fan cord is pulled up through so it won't touch the heater or get caught in the fan. Now place the thermometer in the lid and screw the end of the thermostat clockwise until the fan comes on (or counterclockwise if you go all the way and nothing happens the other direction.) Make adjustments with the screw until the proper temperature is maintained for 24 hours. Now the incubator is ready for placing in the eggs.

Always have the eggs at room temperature before placing them in. Write the date you place them in on the egg with pencil. Eggs can be kept for about 2 weeks at 10 degrees C if they are turned each day. A regular refrigerator kills them. Turn the eggs in the incubator once a day, you can easily tell when they are turned by using pencil to make a big X on one side of the egg, keep the water dish replenished. The moisture

is most important at the time of hatching. Try not to open the lid the last day (and don't turn the eggs after they pip a hole), as that decreases the humidity and they will not hatch. You will hear them if they are hatching. Before hatching you will have to place a barrier in front of the fan so the chicks don't walk into it, such as chicken wire. I meant this incubator to be for early development only, but it could be altered in this way to use to hatch as well. Incorrect temperature or too low humidity can cause death during development.