Lab Handout: The Effect of Caffeine on the Heart Rates of Three-Day Old Chick Embryos

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Objective: The purpose of this experiment is to observe the effect of caffeine on the heart rate of three-day old chick embryos by directly treating them with three different concentrations of caffeine.

Background: Mammalian embryos are exposed to many of the same chemicals as their mothers through their placenta (Gilbert, 2003). Should a pregnant woman ingest caffeine, the embryo would also be affected. However, unlike an adult, embryos do not yet contain the liver enzymes to degrade caffeine (Braun, 1996.). Therefore caffeine in particular has the potential to inflict irreversible harm on a developing embryo.

The first functional organ of both chick and human embryos is the heart (Gilbert, 2003). The heart is obviously vital to further development. Chick embryos at three days and five-week human embryos both have a tubular heart structures with one atrium and one ventricle (Gilbert, 2003). The hearts of three-day old chick embryos, when removed and exposed to various levels of caffeine will respond similarly to human embryonic hearts under the same conditions. This experiment examines the effective range of caffeine concentrations on the heart rates of embryonic chicks that are three days old.

Preparation:

1) Warm Dulbecco's Eagle Medium and Howard Ringers solution to 37 degrees C.
2) Pour caffeine solutions and DEM into four 2ml plastic dishes:

To make caffeine solutions: (With the stock 1mg/ml caffeine solution in DEM, the following instructions make enough of the proper caffeine solutions for four participants)

A) Add 6 ml of caffeine and 14 ml of DEM (total: 20 ml of 0.3 mg/ml caffeine).

B) Add 4 ml of caffeine solution and 16 ml of the DEM (total: 20 ml of 0.2-mg/ml caffeine).

C) Add 2 ml of 1.0 mg/ml Caffeine solution to 18 ml of DEM (total: 10 ml of 0.1 mg/ml caffeine).

3) An additional 2 dishes should be filled with warmed Howard Ringers solution to perform dissections and to use as additional group controls.
4) Make a chart for recording the heart beats that includes the following information:

Egg #	Howard Ringers solution	Base (Eagle medium)	0.1 mg/ml Caffeine	0.2 mg/ml Caffeine	0.3 mg/ml Caffeine
#1 (Beats per minute)					

5) Take one egg out of the incubator and place in Styrofoam tray on the bench.

Extraction of the embryos:

6) Clean the surface of the egg with 70% ethanol.
7) Puncture the wide end of the egg using dull forceps, remove the top of the shell and the shell membrane.

8) Grasp the embryo with the blunt forceps. Using a spoon, remove the embryo from the egg and place it in a dish of Howard Ringers solution.

Removal of the heart:

9) Using the fine forceps, remove the trunk region of the embryo above and below the heart.
10) Remove the heart region, leaving the heart intact and place the heart in a dish of Howard Ringers solution.
11) Try to record at least 3 or 4 hearts.
12) The beats of each heart should be recorded for a minute in one of the solutions containing caffeine and the controls.

Literature Cited

Braun, Stephen. 1996. Buzz: The Science and Lore of Alcohol and Caffeine. New York: Oxford University Press. pp 155-156.

Gilbert, Scott F., 2003. Developmental Biology, 7th ed. Sinauer Associates Inc. Publishers. Sunderland, Mass. pp. 492-8.