

Effect of Caffeine on the Heart Rate of 3- or 4- Day Old Chick Embryos

1. **Objective:** The purpose of this experiment is to observe the effect of different caffeine concentration on the heart rate of three day embryos. The difference caffeine concentrations are 0.25 mg/ml, 0.5 mg/ml, and 1.0 mg/ml.

2. Background –

In development of a chicken embryo, the heart is the first functional organ to develop. This experiment utilizes 3- or 4- day embryos where the heart is developed into a tubular structure, and is actively pumping fluid. However, it is not yet regulated by the nervous system. As the only functional organ in the embryo, the heart initiates its own contractions using a sodium calcium gradient. External, foreign chemicals can disrupt this gradient and result in damage to the circulatory system. Activity of cardiac muscles in early development is regulated solely by the sodium and calcium ion channels. Depolarization occurs following an influx of these ions, which creates the action potential. Potassium ions then exit the cell, causing repolarization and this cycle repeats in healthy individuals.

Caffeine is a teratogen in embryo development, meaning that it can lead to developmental abnormalities and cells damage. Caffeine has also been shown to increase diastolic volume, stroke volume, and cardiac output, all of which contribute to increased heart rate (Byuere 1987). Caffeine has also been shown to stunt growth in all organs of the chick embryo. As the concentrations of caffeine are increased, the damage to organs is increased as well. Along with sufficient damage to the circulatory system and other organs, caffeine causes neural tube defects (Lee 1982).

3. Procedure –

List of materials:

- 3- or 4- day old chick embryos (4-6 per group)
- Stock Solution Caffeine (1.0 mg/ml) to make (0.25 mg/ml and 0.5 mg/ml solutions in DMEM or Howard's Ringers solution)
- Incubator
- 70% Ethanol
- Styrofoam Egg Cartons
- Dissection Kit:
 1. Blunt Forceps
 2. Two pairs of fine forceps
 3. Small Scissors
 4. Plastic Spoon
- Howard's Ringers Solution
- Sterile 60mm Petri Dishes (4)
- Stopwatch/Timer

- Dissection microscope
- Dulbecco's Modified Eagle's Medium (DMEM) – optional

Note: A source of glucose (e.g. DMEM does not appear to be necessary for intact embryos, but may be necessary to obtain reproducible results with isolated chick hearts.

Detailed protocol:

1. Dilute the 1.00 mg/ml stock caffeine solution to final concentrations of 0.25 mg/ml and 0.5 mg/ml.
2. Pour Howard's Ringers solution in 1 petri dish for the control.
3. Pour 0.25 mg/ml, 0.5 mg/ml, 1.00 mg/ml concentrations of caffeine into the other 3 petri dishes.
4. Remove the eggs from the incubator and place them on the styrofoam egg cartons.
5. Consult the Basic Protocol for the extraction of the chick embryo using the dissection kit.
6. Transfer one embryo into each petri dish with the Howard's Ringers solution and measure the heart beat in one minute.
7. Then transfer the same embryo into the lowest concentration of caffeine (0.25 mg/ml).
8. Immediately place the petri dish under the dissecting microscope.
9. Measure the heart rate of each embryo for 1 minute using the stopwatch.
10. Take the second chick embryo place it in the Howard's Ringers solution and measure the heart beat in one minute and move it into the caffeine solution concentrated at (0.50 mg/ml).
11. Repeat step 10 with the third embryo, placing it into (1.0 mg/ml) caffeine solution to measure the heart beat.
12. Do steps 6-10 for the other set of embryos for second trial.
13. Record the data into the data sheet.

Works Cited

Bruyere, Harold J., Bernard J. Michaud, Enid F. Gilbert, and John D. Folts. "The Effects of Cardiotoxic Doses of Caffeine on Cardiac Function in the 3-day Chick Embryo." *Journal of Applied Toxicology* 7.3 (1987): 197-203. Print.

Lee, H., R. G. Nagele, and J. F. Pietrolungo. "Toxic and Teratologic Effects of Caffeine on Explanted Early Chick Embryos." *Teratology* 25.1 (1982): 19-25. Print.

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