DETERMINING THE EFFECTS OF ALCOHOL ON THE 5-DAY CHICKEN EMBRYO HEART RATE

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Abstract:

In this experiment a 5-day chicken embryo was selected to study the effects of alcohol on the embryonic heart rate. This was done by windowing the egg and taking the *in vivo* heart rate of the embryo. Then the embryo was explanted from the shell and placed in a Syracuse dish where the heart rate was taken *in vitro* using a dissecting microscope and a stopwatch. Finally, 0.0002%, 0.002%, 0.02%, and 0.2% alcohol concentrations were added to the embryo and the heart rate was recorded three times after each application. As the concentration of alcohol added to the embryo increased, the heart rate of the embryo decreased or entered cardiac arrest. In this experiment, several negative effects that alcohol had on the embryonic heart were also noted. In several of the embryos there were severe cases of atrial flutter and tachycardia which caused the heart rate to be much faster and more of a random pace. From previous research it is reasonable to say that alcohol affected the sinus venosus by disrupting its "pacemaker potential" (3). Once this had happened the hearts were never able to regulate themselves normally and produced very random heart rates. Overall, the application of alcohol to the embryonic 5-day chicken heart *in vitro* had very harmful effects to the heart rates and the embryo as a whole.

Introduction:

The chick has been chosen for this experiment as a "model system" since it has a four-chambered heart and similar developmental patterns to that of the human heart. Basically, between 25 and 30 hours of incubation the paired heart vesicles begin to fuse from the anterior to posterior end. This fusing forms one continuous mesenchymal tube that has four parts: conotruncus, ventricle, atrium, and sinus venosus. At approximately 33 hours the mesenchymal tube bends to form an S-shaped bend. By 48 hours the heart folds over itself, forming a single loop. After 72 hours the atrium expands in preparation for turning into the left and right atria. The heart begins to beat after the paired heart rudiments have finished fusing, before the conotruncus forms. After the heart tubes have fused, the sinus venosus begins to act as the embryonic pacemaker, later being incorporated into the right atrium (6).

Alcohol has been shown to directly affect the heart and blood vessels in humans (3). High levels of drinking cause cardiac arrhythmias such as tachycardia, fibrillation, and atrial flutter. All fetuses can be directly affected by alcohol *in vivo* by the ingestion of alcohol by a parent. Ethanol's impact on cardiac development is also influenced by fetal genetics (4). Other research has shown that if using baby chicks, a noticeable difference in beak length, head diameter and body size can be seen (5). Herein, it is hypothesized that alcohol will cause a decreased heart rate in the 5-day chicken embryo and will eventually cause the embryo to enter cardiac arrest.

Materials and Methods:

I. Preparation of Alcohol Solution

The first step of this experiment was to create a serial dilution of alcohol. Using a 2% stock alcohol solution and chick saline, a serial dilution was used to produce 0.2%, 0.02%, 0.002%, and 0.0002% alcohol concentrations.

II. Windowing an Egg

The methods of Cruz et al, 1993 were used to window eggs. Basically, Scotch tape was placed on one half of an egg, and then using scissors, a hole was poked near the bottom portion of the egg. Then a syringe was used to remove excess yolk, and an oval opening was cut in the shell to expose the embryo. The *in vivo* heart rates were taken three consecutive times at fifteen seconds each using a stopwatch.

III. Extraction of Embryo

The methods of Cruz et al, 1993 were used to extract the embryo from the egg. A filter paper "doughnut" was placed around the embryo and was allowed to sit for 15 seconds, allowing the doughnut to adhere to the vitelline layer. Then, using fine scissors, the vitelline layer was cut around the doughnut and removed from the egg using a spoon. The embryo was then placed into a Syracuse dish that was filled with warm chick saline. The solution was changed periodically to ensure that it stayed clean and warm. The *in vitro* heart rate was then taken three consecutive times at fifteen-second intervals each.

IV. Administration of Alcohol

The final portion of this experiment was the exogenous application of alcohol to the embryo. First the 0.0002% concentration of alcohol was applied to the embryo and allowed to absorb into the embryo for 30 seconds. Then the *in vitro* heart rate was taken three times at 15-second intervals. This was then repeated for the 0.002%, 0.02%, and 0.2% alcohol concentrations, taking the heart rate three times after each solution at 15-second intervals. This entire process, from windowing to extraction, was repeated for five different embryos.

Results:

It was hypothesized that when the concentration of alcohol was increased, it would cause a decrease in the *in vitro* heart rate of the embryos. The data for five 5-day explanted embryos that were studied are displayed in Figures 1-5. For embryo #1, all three *in vivo* heart rates equaled 80 beats per minute (bpm); after explantation, the *in vitro* heart rates dropped to 60, 52, and 52bpm (Figure 1). With the 0.0002% alcohol concentration the rates continued to gradually drop to 48, 52, and 52bpm. After the application of the 0.002% alcohol the heart rates again dropped to 32, 36, and 40bpm. Once the 0.02% alcohol was added, the heart rates to level out to 48, 40, and 36 bpm.

For embryo #2, the *in vivo* heart rates were high at 128, 120, and 120 bpm (Figure 2). Again the *in vitro* heart rates dropped once the embryo was explanted, and equaled 88, 96, and 68bpm. When the 0.0002% alcohol concentration was added there was a drastic drop in the heart rates to 32, 56, and 64 bpm. The embryo started to show signs of atrial flutter. After the 0.002% alcohol concentration was added the embryo showed signs of tachycardia with heart rates of 128, 112, and 12 bpm. The embryo then entered cardiac arrest before any more concentrations of alcohol could be added.

For embryo #3, the *in vivo* heart rates were high again at 132,116, and 128 bpm (Figure 3). The *in vitro* heart rates slightly dropped again down to 104, 104, and 92 bpm. After the 0.0002% alcohol concentration was added there was another decline in heart rates down to 44, 48, and 52 bpm. After the 0.002% concentration of alcohol was added the heart rates dropped slightly from the higher concentration but leveled out to 40 bpm for all three trials. However, once the 0.02% alcohol was added the embryo started to experience tachycardia, which can be seen by the large increase in heart rates to 128, 112,

and 72 bpm. With the addition of the 0.2% alcohol the heart rates then dropped down very low again and leveled back out to 32, 36, and 36 bpm.

For embryo #4 the *in vivo* heart rates were not measured due to technical difficulties, so the average *in vivo* heart rate for the other four embryos was used which was 100 bpm. The *in vitro* heart rates were 60, 40, and 32 bpm (Figure 4). After the application of 0.0002% alcohol, the heart rates dropped down to 28, 24, and 20 bpm. After the application of the 0.002% and 0.02% alcohol concentrations all of the heart rates tended to level out with rates of 24 bpm for all six of the trials. With the application of the 0.2% alcohol the heart rate still maintained a fairly low but constant heart rate with 24, 20, and 24 bpm.

For embryo #5 the *in vivo* heart rates were 80, 60, and 60 bpm (Figure 5). Once it was explanted from its shell the *in vitro* heart rates were measured at 88, 76, and 52 bpm. With this embryo, as soon as the alcohol was added the heart rates dropped drastically and the embryo started showing signs of atrial flutter and tachycardia almost immediately. The heart rates after the 0.0002% alcohol was added were 20, 12, and 8 bpm. Then the heart rate jumped back up quickly and dropped again with the application of 0.002% alcohol, which gave heart rates of 44, 4, and 8 bpm. When the 0.02% the embryo nearly entered cardiac arrest but it still maintained a weak heart rate with 0, 28, and 0 bpm. Once the 0.2% alcohol concentration was added atrial flutter could be seen much better with heart rates of 12, 0, and 40 bpm.

Figure 6 plots the *in vivo, in vitro*, and alcohol affected heart rate averages for the five 5-day embryos. It is evident that the *in vitro* heart rates declined following explanation, and that alcohol at all concentrations tested decreased the heart rates even

further. It should be noted that various arrhythmias occurred throughout the exposure to alcohol, as did one episode of cardiac arrest.

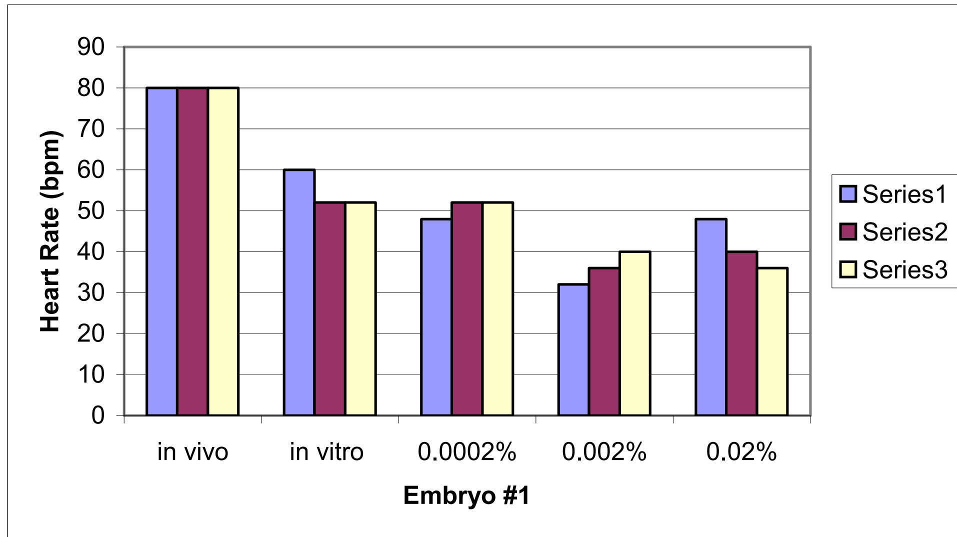

Figure 1- A histogram plotting the *in vivo*, *in vitro* control (without alcohol) and experimental (with alcohol) data for chicken embryo #1. The different concentrations of alcohol were 0.0002%, 0.002%, and 0.02%. The *in vivo* and *in vitro* heart rates differed by approximately 30 bpm. The heart rates of this embryo in the designated alcohol solution fluctuated slightly around the *in vitro* heart rates.

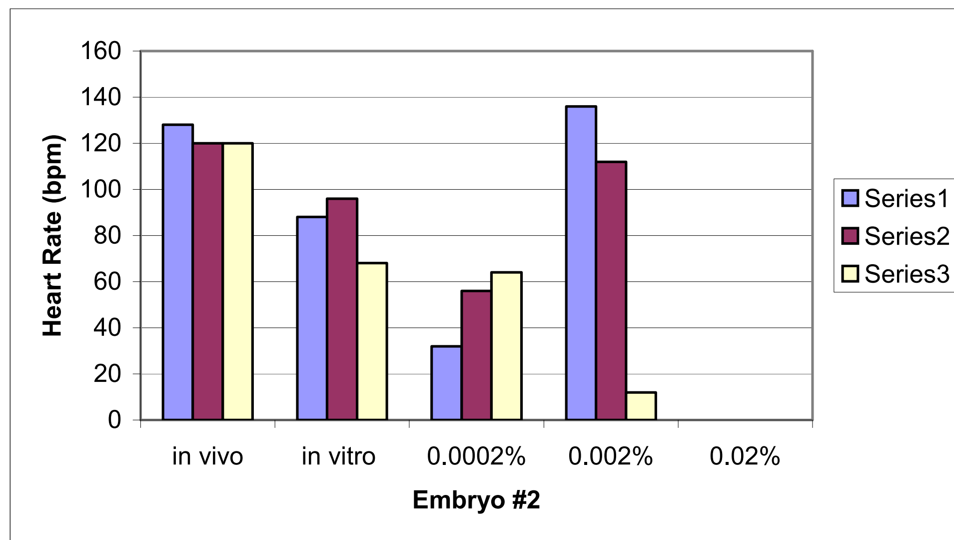

Figure 2- A histogram plotting the *in vivo*, *in vitro* control (without alcohol) and experimental (with alcohol) data for chicken embryo #2. The different concentrations of alcohol were 0.0002%, 0.002%, and 0.02%. The *in vivo* and *in vitro* heart rates differed by approximately 40-60 bpm. The heart rates of this embryo in the designated alcohol solution fluctuated greatly around the *in vitro* heart rates. The embryo entered cardiac arrest with the application of 0.02% alcohol.

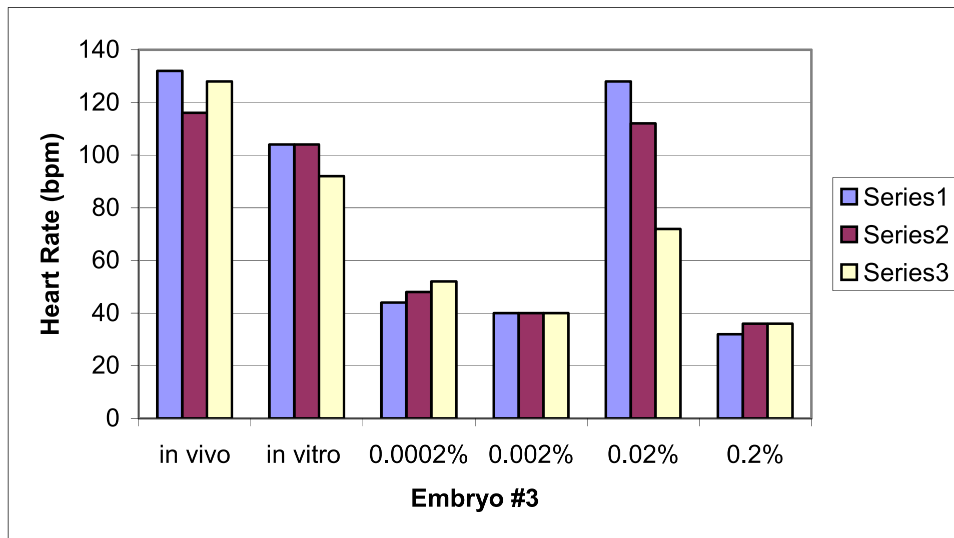

Figure 3- A histogram plotting the *in vivo*, *in vitro,* control (without alcohol) and experimental (with alcohol) data for chicken embryo #3. The different concentrations of alcohol were 0.0002%, 0.002%, 0.02%, and 0.2%. The *in vivo* and *in vitro* heart rates differed by approximately 20-25 bpm. The heart rates of this embryo in the designated alcohol solution dropped much lower than the *in vitro* heart rates but then raised back up above the *in vitro* rates with the application of the 0.02% alcohol concentration.

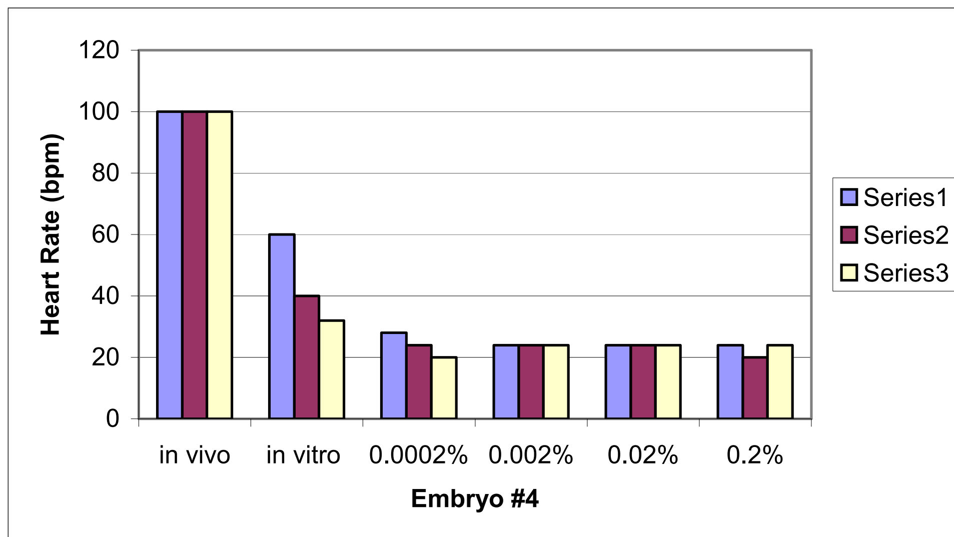

Figure 4- A histogram plotting the *in vivo*, *in vitro* control (without alcohol) and experimental (with alcohol) data for chicken embryo #4. The different concentrations of alcohol were 0.0002%, 0.002%, 0.02%, and 0.2%. The *in vivo* and *in vitro* heart rates differed by approximately 30-60 bpm. The heart rates of this embryo in the designated alcohol solution dropped much lower than the *in vitro* heart rates.

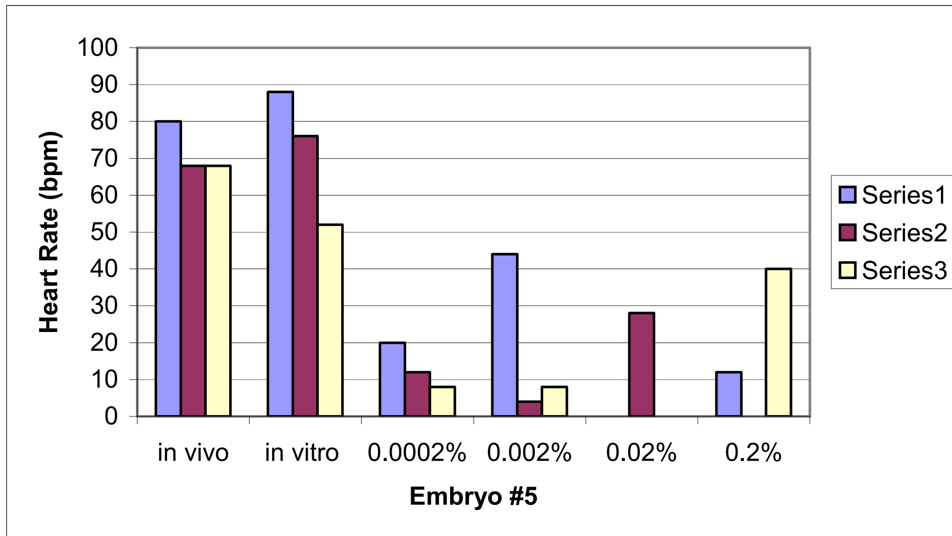

Figure 5- A histogram plotting the *in vivo*, *in vitro* control (without alcohol) and experimental (with alcohol) data for chicken embryo #5. The different concentrations of alcohol were 0.0002%, 0.002%, 0.02%, and 0.2%. The *in vivo* and *in vitro* heart rates differed by approximately 10-20 bpm. The heart rates of this embryo in the designated alcohol solution fluctuated greatly around the *in vitro* heart rates

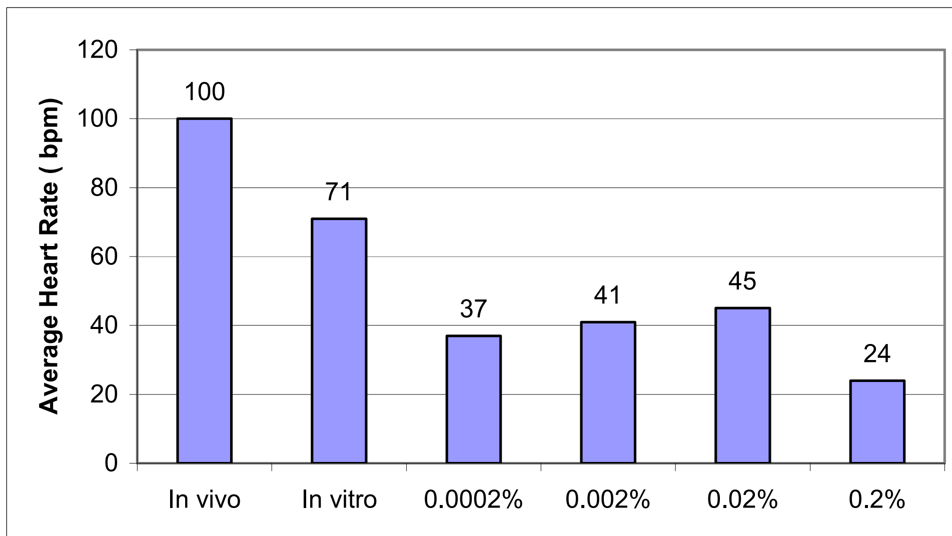

Figure 6- A histogram plotting the average *in vivo*, *in vitro* control (without alcohol) and experimental (with alcohol) data for all five chicken embryos. The different concentrations of alcohol were 0.0002%, 0.002%, 0.02%, and 0.2%. The average heart rates dropped about 30 bpm below the average *in vitro* heart rates.

Discussion and Conclusion:

In this experiment it was hypothesized that the exogenous application of alcohol to the 5-day chicken embryo would cause the heart rate to significantly decrease and would eventually cause the embryo to enter cardiac arrest. This hypothesis was supported by our data because as the embryos were exposed to greater concentrations of alcohol their heart rates dropped much lower than their original *in vivo* and *in vitro* heart rates. Also, cardiac arrest was noted in one embryo.

However, after the 0.002% concentration of alcohol was added, the average heart rate of all the embryos increased slightly. This was due to noted cardiac arrhythmias such as atrial flutter and tachycardia, which were present in several of the embryos. This can be seen in chicken embryo #3 where there was a large drop in the heart rates as soon as the 0.0002% alcohol concentration was added, remaining constant through the 0.002% concentration. Then with the 0.02% alcohol concentration, the heart rates made a huge jump back to approximately the same rate as the *in vivo* heart rate. At this point the effects of atrial flutter were quite evident because the heart would beat very rapidly for a few beats, then it would slow down to a near stop.

In this experiment there were several factors that could have affected our results. The temperature of the chicken embryos was not monitored which could have caused some of them to get colder, resulting in cardiac arrest or slower heart rates. Also, the doses of alcohol were not measured out to be exact, which could have affected the heart rates of the different chicks. For future experimentation, the embryos will be kept in a more closely monitored environment and the experiment will be performed using lower concentrations of alcohol. Previous research has shown that baby chicks exposed to

alcohol show noticeable differences in beak length, head diameter, and body size (5). Based on this information, alcohol will be applied to chick embryos *in vivo* while they are still enclosed in their shell and attached to their extra-embryonic membranes. The embryos will then be explanted from their shell at a later stage of development and compared to embryos of the same age that are not exposed to alcohol.

References:

1. Cruz, Y. P. 1993. *Laboratory Exercises in Developmental Biology***.** Academic Press. San Diego, California. [ISBN 0-12-198390-0]

2. Morgan, J.G. and Brown Carter, M.E. *Investigating Biology: A Laboratory Manual for Biology.* California: Benjamin/Cummings Publishing Co., Inc. 2005.

3. Cavieres, Maria Fernanda and Smith, Susan M. "**Genetic and Developmental Modulation of Cardiac Deficits in Prenatal Alcohol Exposure."** Alcoholism: Clinical and Experimental Research. January 2000. Retrieved February 15, 2006. www.alcoholism-cer.com

4. Kedishvili, Natalia Y. "**cDNA Sequence and Catalytic Properties of a Chick Embryo Alcohol Dehydrogenase That Oxidized Retinol And Hydroxysteroids**." *The American Society for Biochemistry and Molecular Biology*. 272: 7494-7500. <www.jbc.org>

5. Zagory, Jessica. 2004. "**Single Early Dose of Ethanol causes Acute Morphological defects in the chick embryo: A study of Fetal Alcohol Syndrome."** *Developmental Biology*. www.swarthmore.edu/NatSci/sgilber1/DB_lab/Student/FAS.html

6. McLaughlin, J.S. and McCain, E.R. (1997) **"In Vivo and In Vitro Development of the Chicken Heart."** *Tested Studies for Laboratory Teaching,* Volume 19 (C.A. Goldman, Editor). Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education (ABLE) 19: 331-332.