Chick Embryos Exosed to Varied Concentrations of Ethanol and Tyrode's Solution will have Diminutive Facial and Cranial Structures

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Web Posted on December 2, 2004

I. Introduction

Fetal Alcohol Syndrome (FAS) is caused by exposing an embryo to alcohol, which behaves as a teratogen- substance able to stimulate lifelong and profound disabilities, which will in turn affect the developing embryo (Zagory & Yoder, 2004). To study FAS we induced abnormal development by exposing a 72-hour chick embryo to a diluted concentration of ethanol. Ethanol can cause necrosis of the cranial neural crest cells, which are known to later give rise to facial and cranial structures (Smith, 1997). Introducing Ethanol during the period of accelerated cell differentiation and proliferation-critical period- will be more detrimental to the chick embryo (Armstrong, et al., 2004).

Ethanol exposure has been estimated to affect 1 in 1000 human births and to have less apparent effects in 3-4 in 1000 human births (Zagory & Yoder, 2004). A few of the dysmorphologies that can result are: short palpebral fissures (short eye openings), narrow forehead, decreased number of neural crest cells and microcephaly (small brain) (Bupp-Becker & Shibley, Jr., 1998). It has been shown that the effects of FAS in mouse and chick embryos are comparable to the effects on human embryos; therefore the mouse and chick are valuable models to study the effects of alcohol on embryonic development (Zagory & Yoder, 2004). I studied the development of the brain, and more specifically, the development of the eyes due to the invagination of the diencephalon (Wilt & Hake, 2004). The formation and dimensions of the chick’s eyes are important to study due to the straightforward effects the Ethanol may cause upon that specific tissue. To study the dimensions of the brain could be a complicated and at times inaccurate experiment, so by studying the size of the eye one can infer that the ethanol is not only affecting the brain but also in turn affecting the eyes (Bupp-Becker & Shibley, Jr., 1998). In this study we tested the hypothesis that if we introduce Ethanol into a 72-hour chick embryo, then the ethanol will cause the eyes of the embryo to be diminutive and in turn the brain will be diminutive as well.

In this study, we explanted six chick embryos; two will be the controls, two will be exposed to 0.002% Ethanol and the last two will be exposed to 0.02% Ethanol. Due to the fact that FAS is one of the most common birth defects known to the Western world, 3-4 in 1000 human births, studying the effects of alcohol on non-human models may in the long run help to illuminate some of the mystery behind Fetal Alcohol Syndrome and allow for prevention of this avertable disease (Yoder & Zagory, 2004).
II. Materials and Methods

The experiment began by wiping down the lab bench, any surrounding equipment, the paper plate (allowed to air dry) and the experimenter’s hands with a 70% Ethyl Alcohol Concentrated solution. The eggs were then disinfected by wiping them with the 70% Ethyl alcohol and a paper towel, and then the eggs were sprayed with the ethyl alcohol and allowed to air dry with the egg’s wide end down. Next, being very careful, the Chef’s method was used to crack the egg on the lab bench. The egg was then separated into the two halves of the outer shell membrane; ensuring the yolk sac was not punctured! The entire experiment was conducted on top of a sterile paper plate. Next, the contents of the fertilized egg were allowed to slide into the readily available weigh boat, which was set up within the Petri dish on top of the sterile paper plate. Then, before the lid of the Petri dish was applied, using the larger suction pipette and pipette bulb the excess albumen was extracted as to avoid a liquid seal between the edges of the weigh boat and the inside of the lid of the Petri dish. The Petri dish lid was replaced and the contents were allowed to settle. The procedure up till this point was repeated for the five eggs that remained. Prior to using Chef’s style dissection a permanent marker was used to label all of the Petri dishes (top and bottom) with the names of the experimenters and the contents of the dish.

Using a 1 mL transfer pipette 0.5 mL of the Penstrep mixture, containing Penicillin and Streptomycin, was transferred into all six fertilized eggs. The final working solution was 5mL of 2.0% EtOH for the entire class, but 0.5mL per individual egg. An average egg holds about 50mL of albumin. Using the ratio of 0.5mL of 2.0% EtOH to 50mL of albumin (1:100) to determine the final concentration of the EtOH, the outcome was 0.02% EtOH. Then, the final working solution of 0.2% etanol was 5mL of 0.2% EtOH for the entire class, but 0.5mL per individual egg. An average egg holds about 50mL of albumin. Using the ratio of 0.5mL of 0.2% EtOH to 50mL of albumin (1:100) to determine the final concentration of the EtOH, the outcome was 0.002% EtOH. Using a pipette we transferred 0.5mL of the 0.02% EtOH to the correct Petri dishes. Then, also using a pipette the 0.002% EtOH was also transferred to the correct Petri dishes. As a control, 0.5mL of Tyrode’s solution was added to the control dishes. To avoid any contamination careful safety measures were followed and only one pipette was used for each concentration. Before replacing the lids of the Petri dishes, the dishes were checked for any spilled albumin, which could have caused a liquid seal and contaminated the embryos. Carefully, all six eggs were transferred to a 37 incubator. Daily observations were made at the same time of the day using the same microscope (if at all possible). Digital images were taken and saved for future use. The data sheets provided were completed, along with recorded observations relating specifically to the research topic. The experiment was conducted for seven days, then repeated in its entirety so a second trial was also available. The data was collected in Microsoft Excel worksheets so there would be easy access and easy analysis. A Nikon DAGE-MTI DC 200 dissecting microscope was used. Once digital images were taken, a digital image of a ruler was taken and then how many millimeters were viewable at every magnification was determined. By taking the number of pixels in the largest screen and dividing it by the number of viewable millimeters, then the result was 1 millimeter per 1040 pixels for 1.0 magnification, for example. Then, with the digital images on the BTV Pro for OS X software the images were copied and pasted into Adobe Photoshop 7. On Adobe Photoshop 7, the exact region of the image that had to be calculated (i.e. diameter of chick eye) was cut and placed into a new Adobe document. Using the “Image” option the pixels of the cut image were retrieved and then converted to millimeters using the aforementioned conversion determined by using mm/pixels. Lastly, using the entire classes data as a pooled set of data we were able to create a compiled set of data. Using Microsoft Excel two graphs were created: one conveying the diameter of the chicks’ eyes on every day with every concentration and another conveying the body lengths of the chicks on every day with every concentration. Lastly, once the graphs were created one was able to look at them and determine any patterns or trends.

* Adaptation of Armstrong et al., 1994

III. Results

After completing the first trial of our experiment, which included healthy, durable fertilized chick eggs, we found that the embryos exposed to ethanol did have smaller eyes when compared to the controls. However miniscule, there was an indirect relationship between the concentrations of Ethanol and the teratogenic effects the embryos endure. The pattern observed was that the higher the concentration of Ethanol, the greater the effects were on the embryo’s eyes and brain. On day four and day five after incubation, the eyes and brains of the embryos exposed to the concentrations of Ethanol
were diminutive in size.

During the first trial of my experiment both of the 0.02% Ethanol concentrations died after the first day of explantation, one of the 0.002% Ethanol concentrations survived for two days after explantation and only one of the three controls survived until the fourth day after explanation - two out of three died after the first day after explanation. After collaborating with Stephanie Cummings, and being provided with pictures and body lengths by Maya Wolf-Pollina and Natalie Shelton, we were able to combine our data and the outcome was plausible data, which due to our small sample of embryos must be considered preliminary.

The differences in the survivability of the embryos that were exposed to Ethanol and the embryos that were not exposed to Ethanol were extremely dramatic. The pattern of death after the first or second day of explantation was consistent throughout all of the experiments, which were completed by my fellow experimenters. We did not expect such a diminished rate of survivability. The death of almost all of our experimental variables was extremely detrimental to our data collection and severely diminished the size of our data pool. The second trial of the experiments was a disaster as all eggs broke or died shortly after explantation. Due to the condition of the eggs most of the data collected came from the first batch of eggs.

Figure 1. Control Day 4

![Figure 1. Control Day 4](image)

**Figures 1 and 2.** The Day 4 control embryo was pictured at 1.0 magnification it was healthy and developing ideally. The day 5 embryo was pictured at 2.0 magnification. The controls, as compared to Figures 2 and 3, were the only embryos that survived till day 6, had the longest body length and largest eye diameter (Cummings, 2004).

Figure 2. Control Day 5

![Figure 2. Control Day 5](image)
Figure 3. 0.002% Ethanol Day 4
Figures 3 and 4. Figure 2. The Day 4 0.002% embryo was pictured at 1.0 magnification. The vascularization of the Day 4 embryo was much more developed than the day 5 embryo, which was already dead when pictured at 1.0 magnification. The embryos with a concentration of 0.002% Ethanol were measurably smaller in length and eye diameter when compared to Figures 1 and 2 (Wolf-Pollina & Shelton, 2004).

Figure 4. 0.002% Ethanol Day 5
Figure 5. 0.02% Ethanol Day 4
Figures 5 and 6. The embryos with an Ethanol concentration of 0.02% were, in length and eye diameter, the most under-developed. Although, the vascularization was fully developed, the brain structures and eyes were more undersized, in comparison with Figures 1 and 2. Day 4 was pictured at 1.0 magnification; and Day 5 was pictured at 2.0 magnification.

Figure 6. 0.02% Ethanol Day 5
Figure 7. This figure shows the decline in growth of the diameter of the eye when comparing the control, 0.002% and 0.02% ethanol. As the concentration becomes stronger the diameter of the eye developed subsequently becomes smaller.
IV. Discussion and Conclusions

Chick embryos are teratogenically affected when exposed to 0.002% and 0.02% Ethanol. The effects were short body lengths and small eyes. From the data, which was originally very difficult to analyze and form into coherent and feasible data, in the end conclusions could be drawn upon. The conclusions were that as the concentration of Ethanol became stronger, the effects on the chick would progressively become stronger as well. The control embryos were the longest, had the widest eye diameter, which can correlate to the development of the brain because the eyes form from the diencephalon of the brain and lived up to five days longer than the experimental embryos. According to the data collected, the ethanol did cause damage to the brain, which in turn caused diminutive eyes. The body length also appeared to be affected because the toxicity of the ethanol may have caused the developmental process to slow down and in some cases degenerate altogether.

Due to the data that was collectively analyzed, one can conclude that the critical period of the embryological development is within the second day after incubation, and a one-time exposure to ethanol is more than sufficient to harm the embryo at this time. Due to the fact that during the first trial of the experiments 3 out of 4 of the experimental variables died after the first day of incubation, which was a trend throughout the entire classes data, one can also conclude that the Ethanol affected the endurance and survivability of the explanted chick embryos. Also, 2 out of the 3 control embryos did survive until day five. Although, the results show subtle differences in eye diameter and body length, they are still differences that support my hypothesis. These results are preliminary results and I am confident if my experiment was conducted at a larger scale in exact replication, the results would be much more apparent and concrete.
A few sources of error in my experiment were the execution of Aseptic Technique, intrusion of bacteria, damage being caused to the yolk sac and explantation occurring too early. My fellow experimenters and I did attempt to handle the chick embryos as sanitarily as possible, but there is always the chance that an instrument was not cleaned sufficiently or one of the materials was re-handled with unclean hands. The intrusion of bacteria inside the Petri dishes did occur in several of our eggs. After the first day of explantation when my colleagues and I returned to take Day 1 data there would be a small section of the embryo, usually on the corner where bacteria was visibly forming. Bacteria would obviously have detrimental effects on the livelihood of the embryos. Many times during explantation the yolk sac of the egg would be punctured. And although we did question either the professor or the teacher’s assistant about the survivability of those embryos, it is very probable that in combination with the ethanol those embryos were just not able to survive that kind of external mishandling. Explanting the embryos too early was also a source for concern because may be a few of the embryos did begin dividing again, after being in stasis, as quickly as others. If that was the situation, then the embryos were just not ready to live outside of the outer shell membrane and be exposed to all of the bacteria that are constantly diffusing around in air.

If I were to re-conduct this experiment I would do a slew of things differently. I would explant the chick embryos at day 3, be even more cautious while explanting to ensure the yolk sac is not penetrated, strive for a higher level of Aseptic technique and use containers that are sealed more properly than the Petri dishes we used. To extend my results in new directions, experimenters in the future could use one hundred or two hundred fertilized chick embryos. A large problem with my experiment was the sample size. It is nearly impossible to draw upon concrete conclusions when you are dealing with 6-8 embryos. I would also recommend using the same microscopes, but removing the embryo from the yolk so individual brain structures and eye structures can be viewed and manipulated with. The results and conclusions I completed my experiment with, did support my hypothesis, but the experiment should be conducted with a much larger sample so the results will not be considered preliminary.

V. Bibliography

5. Patel, Hemant. Student Collaborator. 2004