

High Insulin Dosages lead to Vascular Defects in Chick Embryos

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Introduction:

The developmental process that I studied in this experiment was the early development of chick embryos exposed to a high dose of insulin. Insulin is “a hormone that regulates the level of sugar in the blood and is produced by the beta cells of the islets of Langerhans in the pancreas. (Britannica, 2007) Insulin is secreted when the level of blood glucose rises, and when it falls secretion of insulin stops, and the liver releases glucose into the blood.” (Britannica, 2007) The regulation of insulin is important because an inadequate production of insulin leads to the condition of Diabetes Mellitus. Diabetes is “a syndrome characterized by hyperglycemia (high blood sugar), but with potentially many causes.” (Zimmet, Walder, Collier. 2006) Diabetes is broken down into two subcategories they are known as Type 1, and Type 2 diabetes because of its causes. In this experiment, I will focus on Type 1 diabetes because it is caused by failure of normal insulin production by the pancreas. (Zimmet, Walder, Collier, 2006) In this study, I tested the hypothesis that embryos with the high dose of insulin will have more developmental complications of the circulatory system due to hypoglycemia than that of the embryos of the non-affected control embryos. It is interesting to study this hypothesis, because I am trying to model a rare case study of acute pulmonary edema caused by insulin overdose-induced hypoglycemia in a patient with type 1 diabetes. (Uchida, 2004) The patient in the case study had injected them self with a massive amount of insulin in order to hurt themselves permanently. Her blood pressure was 160/136mmHg, and her pulse rate was 130 beats per min. These findings were astonishing for me, because the National Heart, Lung, Blood Institute (NHLBI) guidelines define that normal blood pressure are 120/80mmHg, and define normal pulse rate as 60-100 beats per min. (NHLBI, 2003) Although I am not sure if I had induced hypoglycemia nor pulmonary edema in the experimental embryos during the experiments, hypoglycemia is the reduction of the concentration of glucose in the blood below normal levels, commonly occurring as a complication of treatment for diabetes mellitus. (Britannica, 2007) In this study we explanted chick embryos to test this hypothesis in which the experimental chick embryos are exposed to a higher acute level of insulin with a specific concentration of 10mg/ml bovine insulin over a period of 7 days, at a steady temperature of 37°C. Later the results were averaged and the experimental embryos were then compared to the control embryos in order to compare their developmental growths of

target organs in the embryos. The target organs of the embryos that I observed and measured in this experiment are the limbs; such as wing and legs, diameter of the area Vasculosa, width of the main artery, pulse as well as the beat strength of the heart. I decided to focus on pulse rate and beat strength in order to observe any mimic from the case study, since I cannot measure the lungs of the embryos due to lack of resources.

Materials and Methods:

The materials needed to complete this experiment were: viable 33-hour chick embryos, 1 pair of forceps, as many sterile weigh boat dishes, 1 micropipette, Sigma catalog number 19278- 5ml Insulin Solution: Human, 10mg/ml in 25mM, pH 8.2, 1 egg tray, and 70% EtOH. The ICUC is a research laboratory where students can take measurements and make observation thru digital imagery such as movies and captions, of real life frames. The ICUC is equipped with compound and dissecting microscopes, which run the BTV Pro software program that uses a SONY CCD camera to feed the live image onto the computers. The dissecting microscope is the optimal desired tool to use, because it allows you to view the embryo from above. The magnifications used for taking digital images of the embryos were 0.8x and 5.0x. Digital images were captured under the magnification of 1.0x, because at a magnification of 1.0x the ocular units (ou) and millimeters (mm) are equivalent. (i.e. 1ocular unit = 1mm).

I began the experiment by sterilizing the lab bench and tools with 70%EtOH. Next, I gently wiped 1 egg with a paper towel soaked in 70% EtOH and let the egg air dry with the wide end facing down on the egg tray. Next, I cracked the wide end of the eggshell with the tip of the forceps carefully so not to puncture the embryo. By removing the eggshell I created a smooth circular opening the size of the air pocket. Then, I held the egg with its wide end down and poked a hole in the egg membrane to release the embryo into the sterile weigh boat. If the embryo did not exit the shell, I poked a small hole or opening on the narrow end of the eggshell to break the vacuum and release the embryo. The next step was to add penicillin/streptomycin antibiotics to the embryos in order to maintain healthiness. The final step was to label the embryos that were control and experimental. I repeated these steps on all other viable eggs.

(Armstrong, 1994. Lab Protocol. 10/10/07 57-58 R.E.S)

After all the embryos had been explanted into the weigh boats, they were separated into 2 categories. One category was the control and the other was experimental as mentioned before. The embryos labeled control did not receive any insulin, while the experimental embryos did receive insulin. The experimental embryos received 0.25ml of 10mg/ml concentration of human insulin. The experiment is based on a two week period, where the experiment for the controls ran on the first week for 7 days or until viable, and the experimental eggs ran on the second week for 7 days or until viable. On the third week the experiment was over and the data was analyzed. (Lab Protocol, 11/14/07 80 R.E.S)

I returned every 24 hours for 7 days to collect growth and quantitative data. More specifically I measured the pulse rate, beat strength, wing bud and leg bud length, body length, diameter of area Vasculosa, and the width of the main artery. I measured these using a ruler, a timer, a dissecting microscope from the ICUC, a SONY CCD camera, and digital images like film and captions taken during the experiment. I recorded the data on a data sheet. (Tonelli, Dania. 2007) (Lab Protocol, 10/31/07 74 R.E.S)

Results:

The following graphs represent pooled data from all the control embryos of week 1 of the experiment. The data that was averaged together by three embryos called, Crush II, Big Boy, and Porsche. The raw data can be found in the Developmental Lab Manual notebook, Lab# 7, Independent Research Project, (10/31/07 75 R.E.S)

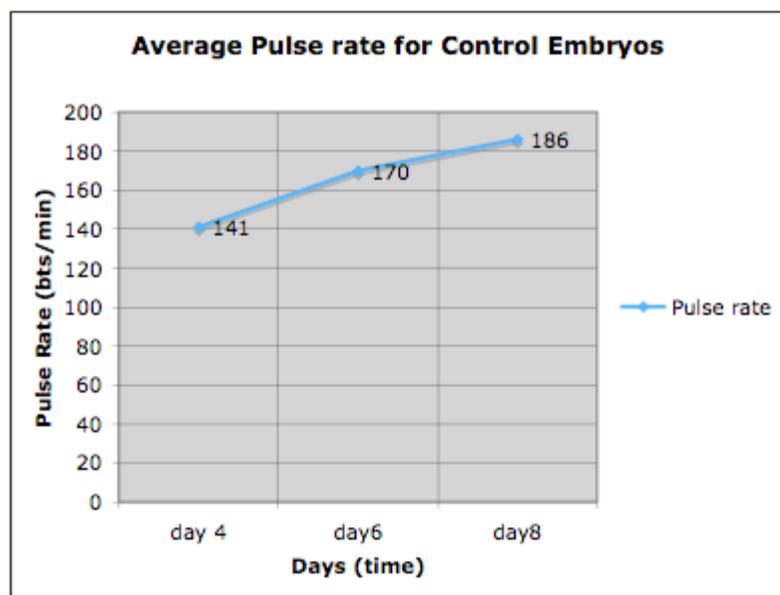


Figure 1.1: Shows the average pulse rate for the control embryos at days 4,6, and 8.

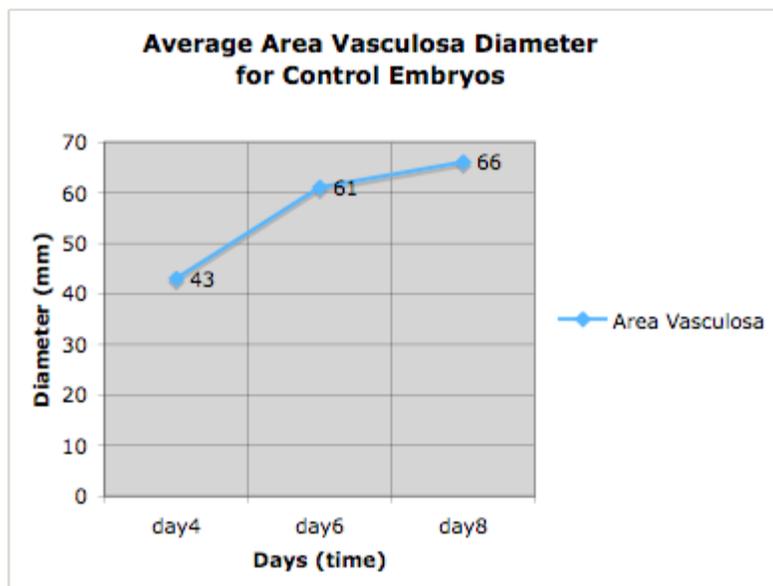


Figure 1.2: Shows the average area Vasculosa diameter for the control embryos at days 4,6, and 8.

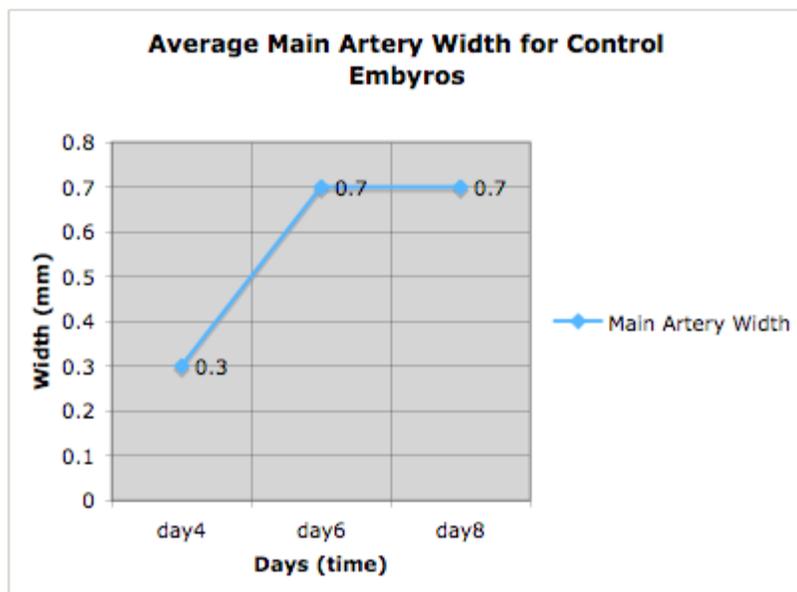


Figure 1.3: Shows the average main artery width for the control embryos at days 4,6, and 8.

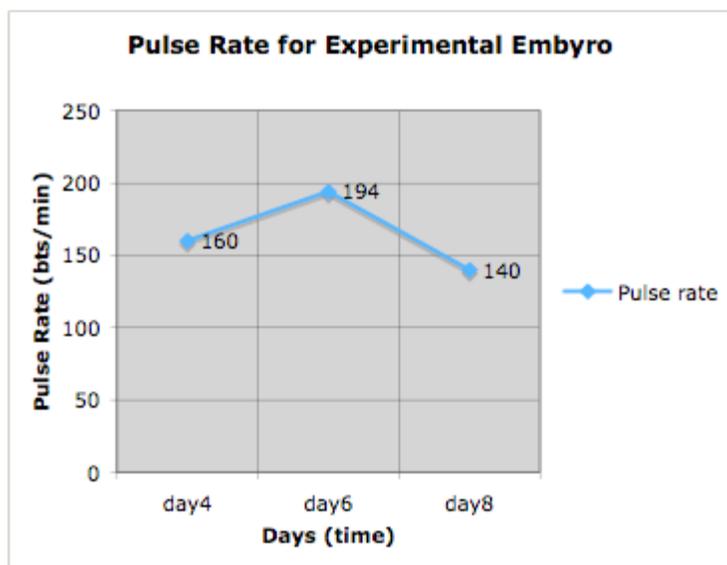


Figure 2.1: Shows the pulse rate for the experimental embryo at days 4,6, and 8.

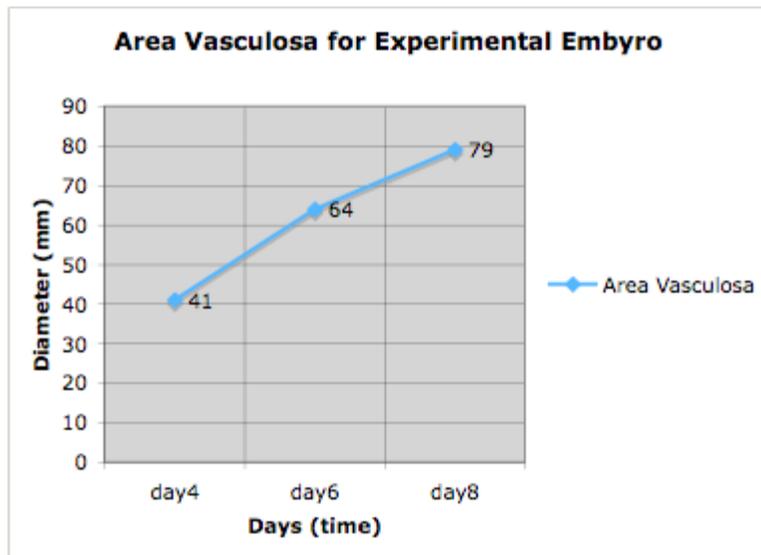


Figure 2.2: Shows the length of the area Vasculosa of the experimental embryo at days 4,6, and 8.

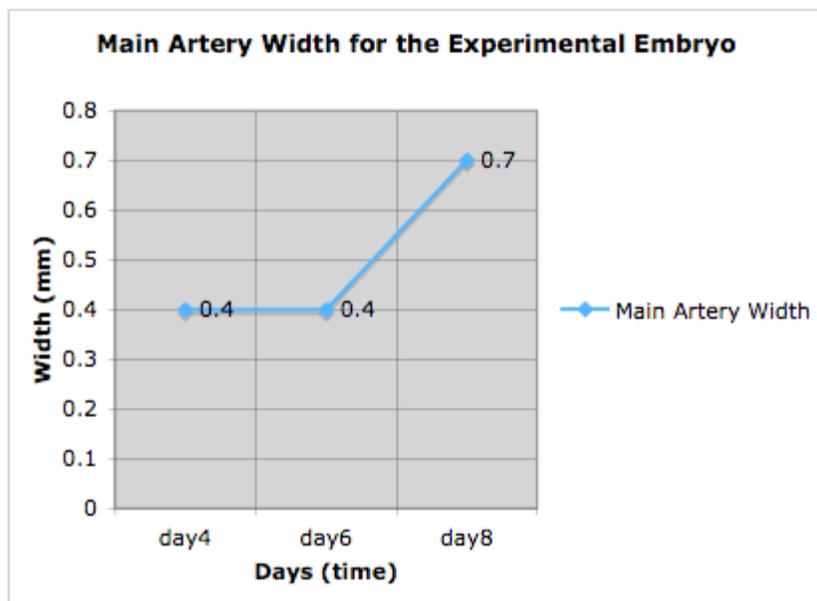
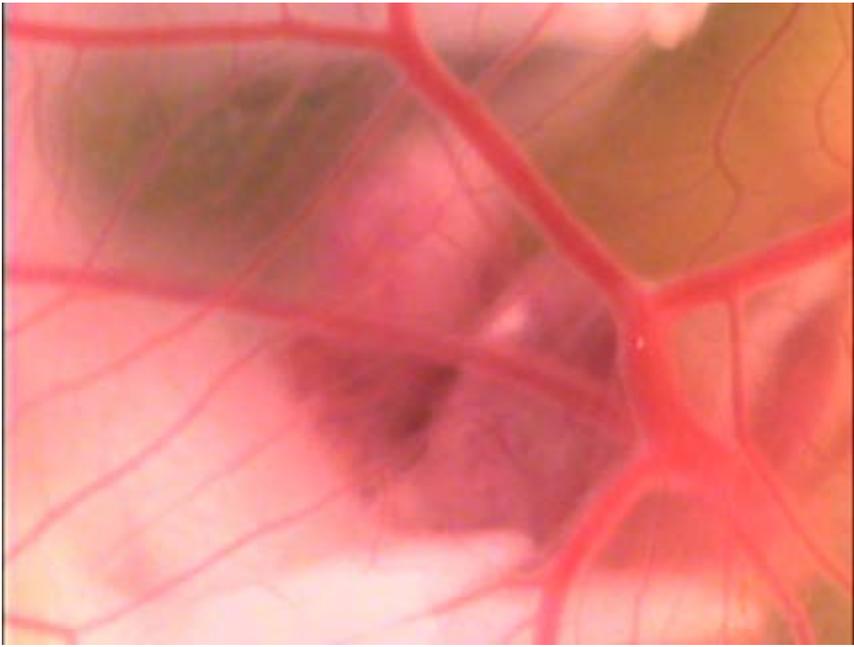


Figure 2.3: Shows the width of the main artery of the experimental embryo at days 4, 6, and 8.

The previous figures 1.1-1.3 all represent the averaged pool data for the control chick embryos of the week 1 period. The graphs show the average growth of vasculature development. The pervious figures 2.1-2.3 represent the only data collected from the only viable experimental embryo. All observations come from Lab Manual, 11/14/07 84-102 R.E.S.



Caption 1.1: Caption is of a control embryo named Porsche at day 8 of development.

Discussion:

The embryo with high insulin exposure had different results than the average data from the control embryos. In my efforts to model the rare case report of deadly high dosages of insulin, the data collected does support my hypothesis. Conclusions that can be drawn from the data are, that the presence of insulin in the early developmental stages of chick embryos, pulse rate is affected. The pulse rate will change and does not remain consistent. The development of the area Vasculosa is surprisingly very similar between the experimental embryo and the average of the control embryos. Which leads me to conclude that, though insulin might affect pulse rate it does not necessarily affect the development of the area Vasculosa. As a matter of fact the area Vasculosa is at each day is greater than the control data, when compared. This may occur because just as much as insulin is a blood sugar regulator it is also a growth factor, or growth protein. (Moses, Tsuzaki, 1991) “There are several other clinical examples in clinical medicine of insulin serving as a growth factor. From an epidemiologic standpoint, hyperinsulinemia, or high insulin dosages, is associated with hypertension and with a propensity to develop macro vascular complications characterized by large arterial smooth muscle proliferation.” (Moses, Tsuzaki, 1991) Hypertension is “also called high blood pressure condition that arises when the blood pressure is abnormally high. Hypertension occurs when the body's smaller blood vessels (the arterioles) narrow, causing the blood to exert excessive pressure against the vessel walls and forcing the heart to work harder to maintain the pressure. Although the heart and blood vessels can tolerate increased blood pressure for months and even years, eventually the heart may enlarge (a condition called hypertrophy) and be weakened

to the point of failure. Injury to blood vessels in the kidneys, brain, and eyes also may occur. (Britannica, 2007) Which explains why the experimental embryo has a different expansion rate of the main artery width. From digital film that cannot be assessed in this paper will show the beat strength of a control embryo and the experimental embryo, as well as the flow of blood at the main artery. These digital images will be presented during the presentation. Beat strength of the heart is directly affected by hypertension, the narrower the artery width the higher the blood pressure will be, and wider the width gets the lower the blood pressure will be. This allows me to conclude that as seen in digital film of blood flow observation, the blood pressure between the control embryos and the experimental embryo are quite different. The digital film and the differences of the pulse rates between the control and experimental embryos can also be seen in the movie of the heart rate.

There were some sources of error during the experiment, the experimental embryo was exposed to a higher dose of insulin than projected, 0.25ml and not 0.1ml. There is only one experimental embryo, thus the results are not full proof. One experimental embryo cannot fully support my hypothesis, it was the luck of the draw in which I was able to see some results. To refine this experiment, a future trail of this experiment would call for more viable experiment embryos. This experiment was based on one viable experimental embryo, thus the data cannot be fully trusted, while having more viable experimental embryos will allow the data to be compared between the experimental embryos and maybe even averaged. Future experiments that would extend my results in a new direction would be, to run another experiment where the embryological categories would be acute and chronic insulin dosages. I would be able to compare the differences if any of the vasculature development.

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