The Affects of Insulin Exposure on the Development of Chick Embryos

Dania Tonelli
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Professor Robert Morris
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INTRODUCTION:

Diabetes is a major disease plaguing the world. Though it may seem unimportant, diabetes mellitus is in the top 10 most significant diseases in the developed world and is gaining in significance. (Diabetes mellitus, 2007) According to the World Health Organization, in 2006, at least 171 million people worldwide suffer from diabetes mellitus, and they estimated that the number would double by 2030. 5-10% of diabetes cases in North America are type 1, while the rest are type 2. (Diabetes mellitus, 2007) Type 1 diabetes requires constant insulin injections, whereas people with type 2 diabetes need less insulin injections or even none at all.

Hypoglycemia is often a complication of diabetes treatment. It is when a person has abnormally low blood sugar. It can happen when a person accidentally injects too much insulin or when they don't eat as much as they had planned. Severe hypoglycemia can cause loss of consciousness, brain damage, and death. (Diabetes mellitus, 2007)

For pregnant, diabetic women, they have to be especially careful to prevent harm to the fetus. Diabetes during pregnancy, whether Type I or Type II, has many effects on the neonate. Approximately 4 percent of pregnancies in the United States are complicated by abnormal glycemic control. (Barnes-Powell, 2007) Maternal diabetes can increase the risk of pre-eclampsia and abnormalities of fetal growth. Also, there is a high risk of macrosomia (when a baby's weight is above a defined limit at any gestational age) because insulin stimulates fetal growth. (Howarth, 2007) Diabetes has profound effects on embryogenesis, organogenesis, and fetal and neonatal growth. Since insulin can be teratogen at abnormal levels, an accidental overdose can cause congenital malformations in the fetus, such as cardiac, musculoskeletal, and central nervous system anomalies. The occurrence of congenital anomalies in infants born to diabetic mothers is approximately two to five times that in the general public, with malformations accounting for a large portion of prenatal losses. (Barnes-Powell, 2007) In severe cases, maternal hypoglycemia can cause spontaneous abortion. Also, the fetus can be born with severe hypoglycemia if the mother has had hypoglycemic episodes during the pregnancy. (Kline, 2006) Diabetics must control diabetes and monitor it throughout the pregnancy to reduce the impact of the disease on the fetus.

For my experiment I will be studying the effects of insulin on a chick embryo. I will attempt to induce hypoglycemia by adding insulin to the embryo. I will be focusing on the wing bud and leg bud formation, and how the formation of these structures is affected by insulin exposure. Since many diabetics choose to have children, it is important to model the affects of maternal hypoglycemia to the fetus in a chick embryo. I chose the chick as a model for this developmental process because they are fairly large animals and have human-like appendages. Since hypoglycemia in the fetus causes congenital malformations, looking at limbs and other structures in a chick will allow me to see these malformations if they so occur. Also, the chick developmental processes are very close to the humans, so the insulin overdose should have similar affects on the chicks as it does on human fetuses.

My hypothesis is that bud formation of a chick embryo will be impeded by acute insulin exposure. I will test this hypothesis by adding insulin to an early chick embryo, in attempt to induce hypoglycemia.

MATERIALS AND METHODS:

The materials I used were: 33-hour chick embryos, 1 pair of forceps, 1 micropipette, sterile weigh boat dishes, 5 ml of 10 mg/ml human insulin solution (Sigma catalogue # 19278), 1 egg tray, 70% EtOH, 1 transparent ruler, 1 clock, 1 dissecting microscope, 1 HP color inkjet printer, 1 Sony CCD camera, and 1 Macintosh G4 running OS X.3 and BTV Pro.

I began the experiment by sterilizing the lab bench and tools. Next, I gently wiped 1 egg with a paper towel soaked in 70% EtOH. I then let the egg air dry with the wide end facing down in the egg tray. Next, I cracked the wide end of the egg with the tip of the forceps, making sure not to puncture the embryo. When I removed the eggshell, I created a smooth circular opening the size of the air pocket. Then, I held the egg, 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 leave the shell, I poked another small hole in the narrow end of the egg to break the vacuum and release the embryo. I repeated these steps with any remaining eggs. (Armstrong, 1994)

For week 1, all viable, explanted embryos were the control group and thus labeled. The viable embryos explanted in week 2 and week 3 were the experimental group, acute exposure of insulin. After labeling all embryos, I added 0.5 ml of solution with penicillin to all the embryos and 0.25 ml of a 10 mg per ml insulin solution to the experimental embryos only. The insulin solution was added onto the chick embryo using a micropipette.

The amount of solution added was calculated using values from previous experiments. Using information from previous experiments, I found that 40-80 IU/kg/day of insulin (Ikegami, 2000) will induce hypoglycemia in mice. I converted this number into millimeters of the 10 mg/ml solution per day to obtain the amount of insulin I would add to

the chick embryos. I began by converting the 40 IU/kg into IU/50g, 50 g being the approximate mass of a chick. I then used a conversion number, 0.0417 (Biochemistry, 2007), to convert that number into mg/ml. I then divided this number by the concentration of the insulin solution to find the dilution necessary. Once I found the dilution of insulin solution (1:120), I found how many millimeters I needed to add in a 50 ml chick embryo. The number I calculated was 0.4 ml of 10mg/ml insulin solution.

I returned every 24 hours for 7 days or until the embryos perished, to collect growth and quantitative data. I quantified the growth of the embryos by measuring the wing bud and leg bud length, body length, and diameter of area vasculosa. I measured these using a ruler and clock. I recorded the data on a data sheet. (Sosa, 2007) This data allowed me to see the differences in growth between the control and experimental embryos. I also took pictures of each embryo on given days at a magnification of 1 using the Sony CCD camera on the dissecting microscopes in the ICUC.

I analyzed my data by comparing the wing bud length and leg bug length of the control group to that of the experimental group. I averaged all the numbers for my 6 control embryos to compare to my single experimental embryo. My experimental controls were the chick embryos without any insulin injected. All the embryos received penicillin, but only the experimental group received the teratogen. This allowed us to truly observe the affects of insulin exposure on the chick embryo because we were able to compare it to a normal chick embryo without any added insulin.

RESULTS:

Measured Leg Bud Length of Embryos (in mm)

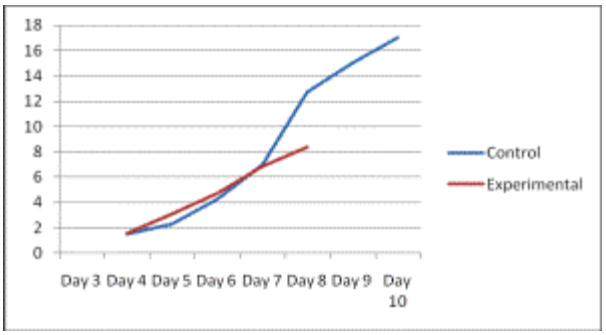


Figure 1- This graph shows the growth of the chick embryos' leg buds over time. It shows the averaged values of the control and experimental embryos on each day. On day 4, six values were averaged; on day 5, five values were averaged; on day 6, four values were averaged; on day 7, four values were averaged; on day 8, three values were averaged; and on day 9 and 10 only one value was used.

Measured Wing Bud Length of Embryos (in mm)

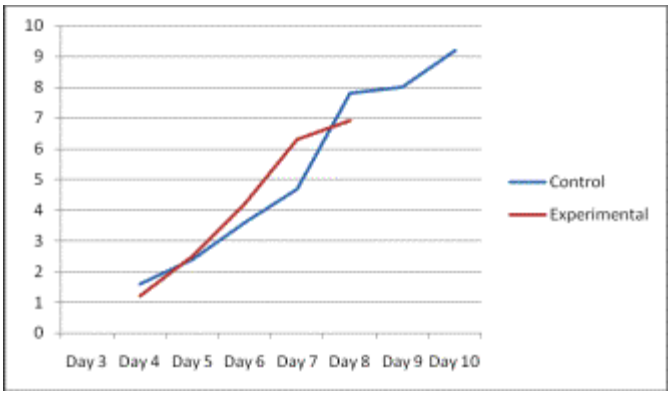


Figure 2- This graph shows the growth of the chick embryos' wing buds over time. It shows the averaged values of the control and experimental embryos on each day. On day 4, six values were averaged; on day 5, one value was used; on day 6, four values were averaged; on day 7, four values were averaged; on day 8, three values were averaged; and on day 9 and 10 only one value was used.

Average Growth of Wing and Leg Buds (in mm) Each Day

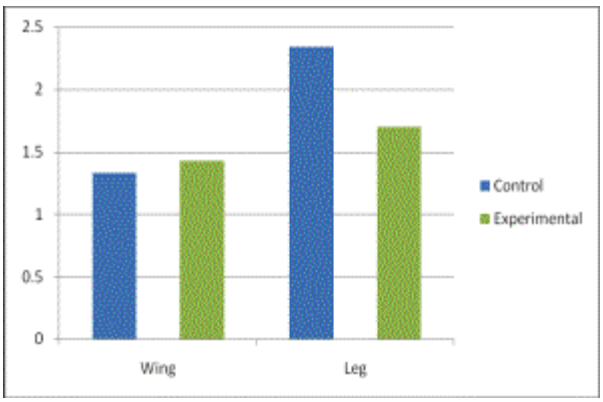
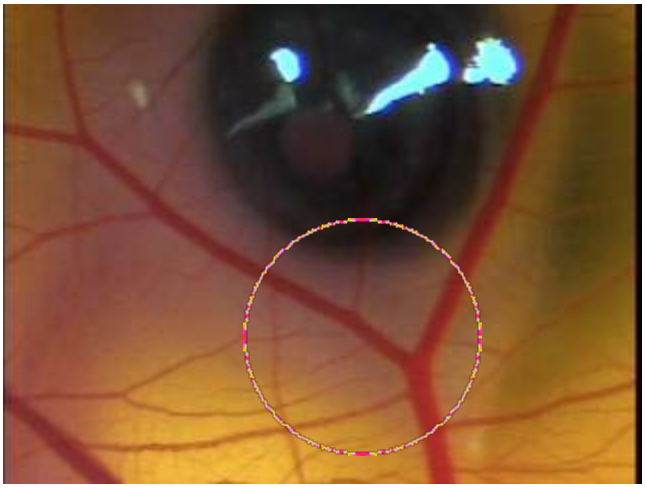
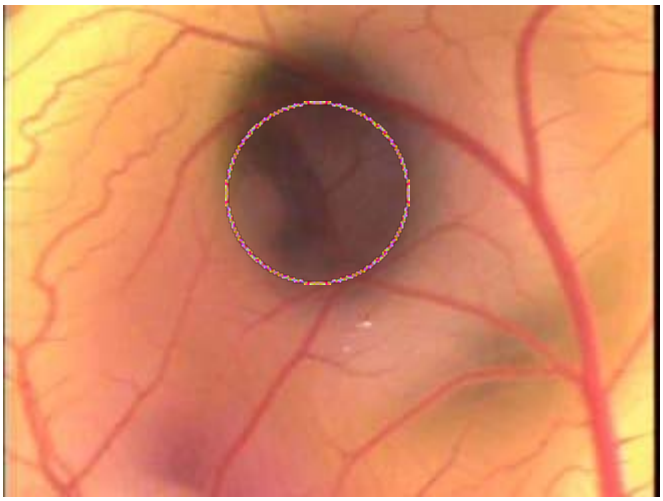


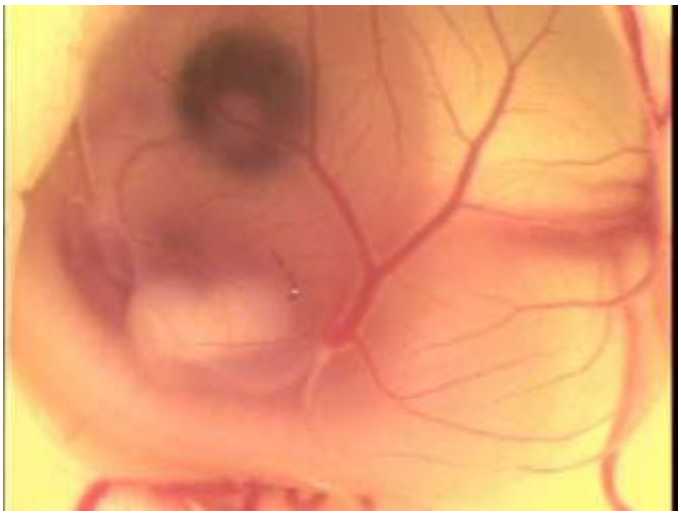
Figure 3- This graph shows the average growth of wing and leg buds each day for the control and experimental groups. Measurements from 5 of the control embryos were averaged.



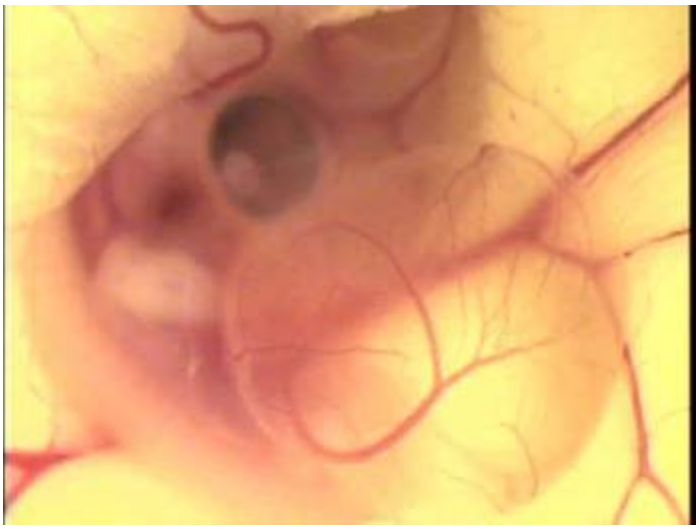
Picture 1- Contrl embryo on day 8 of development. The beak is visible in the circled region.



Picture 2- Experimental embryo on day 7 of development. The beak is visible in the circled region.



Picture 3- Control embryo on day 5 of development.



Picture 4- Experimental embryo on day 5 of development.

Looking at the graphs above, it is clear that there are only slight differences between the control data and the experimental data. The control and experimental groups seemed to grow at the same rate and the same amount each

day. In Figures 1 and 2, one can see that both groups grew about the same amount each day until day 6, with less than a millimeter difference between the two values. Also, the experimental and control groups had more growth on different days. For example, in Figure 2, it is clear that on day 7 the experimental wing bud length was much larger than the control length. However, on day 8, the control group had larger wing bud length. Overall, these peaks in growth seemed to average out over time.

However, the data does show that on day 8, the average length of the leg and wing buds for the control group were higher than that of the experimental group, despite the fact that the lengths had either been equal or lower to the experimental group on day 7. The average leg bud length for the control group on day 8 was higher than that for the experimental group. The average wing bud length for the control group on day 8 was also greater than the length for the experimental group. This information demonstrates the pattern in the data as the embryos age. In Figures 1 and 2, it seems that at the early stages of development, the experimental group had around the same, if not more limb growth than the control group. However, as the embryos reached day 7 and day 8 of development, the control group begins to grow at the faster rate than the experimental group.

In Figure 3, we can see that the average growth per day for the wing buds were very similar between the control and experimental groups. However, the leg bud growth in the control was much different to that of the experimental. The control embryos grew significantly more each day than the experimental embryo.

In Pictures 1 and 2, we can see that the beak of the experimental embryo began to form clearly on day 7 and 8, just as it did in the control embryos. The images of the wing and leg buds (Pictures 3 and 4) show an keen similarity between the control and experimental embryos.

DISCUSSION:

My hypothesis was refuted by my experiment. The data collected did not thoroughly support the hypothesis that leg and wing bud formation of a chick embryo was impeded by acute insulin exposure. There was no strong evidence demonstrating that the growth and development of the leg and wings buds were impeded by the insulin. This could be seen in the results where the growths of the limb buds in the experimental embryo were similar to that of the control group. This can be seen in Figures 1-3 where the growth of the buds was either surpassed by the experimental group or about the same. Though Figure 3 did show that the control group had significantly more leg bud growth per day, this was because the control embryos were able to live longer. As the embryos grew older, the growth per day of the leg buds increased. The experimental embryo was only observed till day 8, so it is uncertain if that embryo may have also

increased in growth per day.

The fact that the control group began to rise above the experimental group in growth around day 8, suggests that the experimental group may have slowed in growth as it continued to develop. The developmental differences between the experimental and control groups may have risen later in development if the embryos had been observed longer. In previous studies, specifically the one by S.K. Basu Ray, the chicks were injected with insulin, unlike my experiment where the insulin was pipetted onto the embryo. The chicks were also observed until day 19, and by this day of development, beak abnormalities, defective body wall formations, and growth retardation were clear in the experimental group. (Ray, 1992) As Ray's experiment suggests, there may have been a divergence in development of the experimental and control chicks if they had continued to develop.

Overall, we can assume that the amount of insulin used, a concentration of 0.05% insulin in the embryo, was not enough to induce developmental abnormalities in the chick embryos. Our hypothesis was proved incorrect, and demonstrated that at that concentration and stage of development, insulin has no clear affect on the development of the leg and wing buds.

The error in this experiment was that more insulin should have been added to the experimental embryos. The literature said that 0.4 to 0.8 ml of 10 mg/ml insulin solution (a concentration in the embryo of 0.08-0.16% insulin) should be added, however, we only added 0.25 ml to the embryo. Since the embryo was our only viable embryo, we did not want to kill it by adding too much insulin. If more had been added, the embryo may have survived and given the experiment different results.

In future experiments there are many changes that I will implement. The embryos will be injected with insulin while they are still in the egg in order to prevent contamination and death due to explantation. I think this will increase the amount of viable embryos at the end of the experiment, however, it will prevent the experimenter from collecting data while the embryo is growing. In order to guarantee the success of this method, a high number of experimental embryos will be necessary and also a large amount of insulin will be necessary. Also in future experiments, I will add more insulin to the embryos. The literature suggested that 0.4 ml to 0.8 ml of 10 mg/ml insulin solution should be used. (Ray, 1992) The increase in insulin will increase the affects of insulin on the chick embryo development. Also, if possible I will observe the embryos for a longer period of time. In order to see the full effects of the insulin, the chicks must be observed past day 10 of development. Finally, in order to protect the viability of the eggs used, the eggs will not sit in 18 °C for more than a couple days. By haulting the eggs natural development processes we are killing and damaging the eggs. The less time the eggs are paused in development, the better.

In order to extend the results of this experiment, different developmental properties should be studied. Instead of

focusing on structural abnormalities that develop in the experimental embryos, the neural development should be studied. The formation of the central nervous system should be observed closely to see if insulin exposure impedes that aspect of embryonic development.

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