Thyroxine Induced Metamorphosis in a Neotenic Axolotl (*Ambystoma mexicanum*): Gills, Lungs, and Capillaries

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Abstract

The Mexican axolotl is a neotenic animal; it reaches full adult size and sexual maturity while retaining its larval characteristics. The axolotl will, however, metamorphose if injected with thyroxine (either T_3 or T_4). During this experiment, Mexican axolotls were injected with T_3. Through the metamorphosis, axolotls were sacrificed at weekly intervals to observe metamorphic alterations as a function of time. Their lungs were removed, preserved, sectioned and placed on slides for viewing with a microscope. The lung volume was then calculated and used as a measurement for potential oxygen and carbon dioxide exchange. It was determined that the lungs of animals treated with thyroxine were more effective for gas exchange as compared to the lungs of control animals. The experimental lungs had a larger lumen, thinner lung walls, greater volume, greater surface area and more vascularization all of which enhance gas exchange.

Introduction

Mexican axolotls (*Ambystoma mexicanum*) are neotenous animals, meaning they fail to complete their metamorphosis. They obtain their full adult size and sexual maturity while retaining larval characteristics such as external gills and a dorsal tail fin. In 1920, J.S. Huxley performed an experiment in which a diet of thyroid glands was shown to induce axolotl metamorphosis. Over three weeks time, Huxley saw a reduction in the axolotl's external gills and the dorsal tail fin, an alteration of skin color and thickness, as well as a strengthening of the limbs to support its body mass on land (Huxley 1920).

The early 1900's contained multiple experiments establishing the connection between the thyroid gland and metamorphosis. It was reported in 1917 by Hoskins that cold-blooded vertebrate animals missing the thyroid fail to metamorphose but still reach normal adult length and weight. Experiments ensued suggesting the administration of either thyroxine or iodine induces metamorphosis in the axolotl. Finally in 1924, Swingle proposed that the axolotl is insensitive to its own thyroid secretions, suggesting an insufficient production of thyroxine or low concentrations of cellular thyroxine receptors (Lynn 1951).

Current research suggests the axolotl is neotenous due to reduced levels of plasma T_4 (DL-thyroxine) as well as reduced levels of thyroid stimulating hormone (TSH). An injection of TSH will increase the plasma level of T_4, suggesting the axolotl is neotenous due to reduced secretions of TSH. With low concentrations of TSH, the animals does not produce active concentrations of T_4 (Galton 1992). Experiments by Prahlad and DeLanney revealed axolotl metamorphosis can be induced by T_4 as well as T_3 (3,3',5-triiodo-L-thyronine) injections (Prahlad 1965).

Prahlad injected axolotls of varying ages with both T_3 and T_4. It was determined that young (120 days old) animals injected with T_3 metamorphose within 14 days. A full metamorphosis is defined by a 100% decrease in gill length. One-and-a-half-year-old animals injected with T_3 metamorphose in approximately 20 days, 15 days faster than those injected with T_4.

The research was designed to quantify a hypothesized increase in oxygen and carbon dioxide exchange potential in the lungs of axolotls treated with T_3 as compared to the lungs of untreated animals. In other words, does the lung of the metamorphosing axolotl undergo anatomical alterations to accommodate for the loss in gill surface area? Observations and measurements to be made include: the cross-sectional area, volume, surface area, vascularization, and wall thickness of both the experimental and control animals' lungs as well as the surfacing habits of the animals.

Materials and Methods

The twenty 3- to 4-month-old animals used in this experiment were obtained from Indiana University Axolotl Colony. The animals were maintained in eight-inch stacking dishes filled 3/4 inch full with Holtfreter's Solution (14.0g NaCl, 0.20g KCl, 0.40g CaCl, 0.80g $NaHCO_3$, 10.0L H_2O). Animals of similar size were maintained together. They were fed every third day with beef liver cut into small pieces. Three hours after feeding, their water was changed and the bowls were cleaned with Alconox and rinsed thoroughly.

The axolotl's kidney contains open nephrostomes at this time of its development. Therefore, any injection of hormone into the pleuro-

Table 1: Data collected from lung cross-sections.

GROUP	D7E	D14E	D21E	D49E	D10C	D21C
Circumferance (CM)	0.30072	0.36690	0.72850	0.62650	0.42010	0.42556
Radius (CM)	0.04796	0.05839	0.11594	0.09971	0.06686	0.06773
Area of Lung (CM^2)	0.00720	0.01071	0.04223	0.03123	0.01404	0.01441
Length (CM)	1.20000	1.00000	1.65000	1.70000	0.80000	1.52000
Volume (CM^3)	0.00864	0.01071	0.06968	0.05309	0.01124	0.02191

peritoneal cavity would be excreted before it could induce metamorphosis. Therefore, the axolotls were injected once with T_3 into the mesenchymal tissue at the base of the dorsal fin with a small gauge needle. The solid T_3 was dissolved in one drop of warm 0.1 M - 0.01 M NaOH. Eventually, 10.0 µg of hormone dissolved in 0.10 mL of 0.60% saline were injected into each axolotl. The control animals were injected with saline.

Surfacing counts were performed before the injections and weekly thereafter as an indication of lung use. Over a ten-minute time, the number of times surfacing was recorded. This was done in experimental as well as in control animals.

External observations were made on a semi-daily basis. The gill reduction, tail reduction, head shape, eye displacement, skin color, limb strength, and time spent out of the water were all noted. Sacrifices were made on a weekly schedule by placing the animals in 0.3% MS222 for a half hour. Sacrificed animals were preserved in a 10% formalin solution. The experiment was run for 21 days with controls; however, two animals were kept alive for a total of 49 days each to observe any alterations occurring in the last four weeks.

The preserved animals were then dissected. The lungs were removed and stored in individual containers with labels. The approximate weight and length of each lung was recorded and external observations were noted. Through routine histological methods, the lungs were embedded in paraffin wax, sectioned with a microtome, and placed on slides. The slides were then stained and observed with a microscope to obtain the circumference of the lung cross-section as well as the thickness of the lung wall.

Results

The sectioned lungs were labeled according to their day of sacrifice (7, 14, 21, 49) as well as their status in the experiment (E for experimental, C for control animals). Their cross-

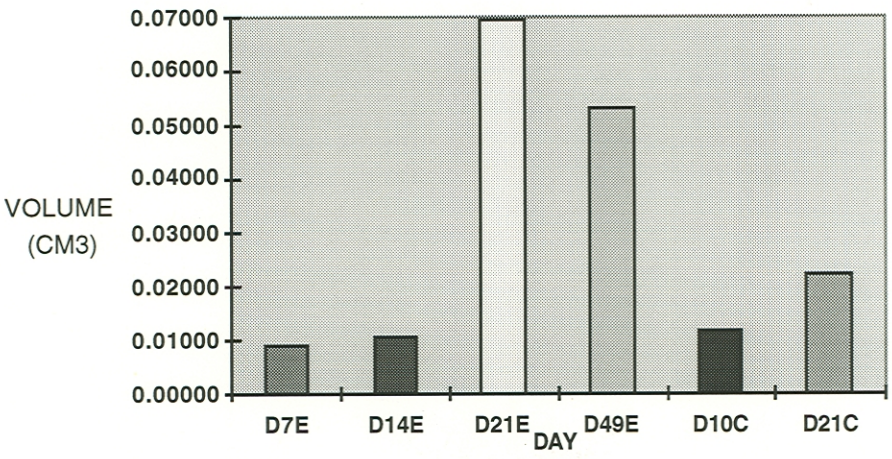

Figure 1: Graphical representation of lung volume as a function of time.

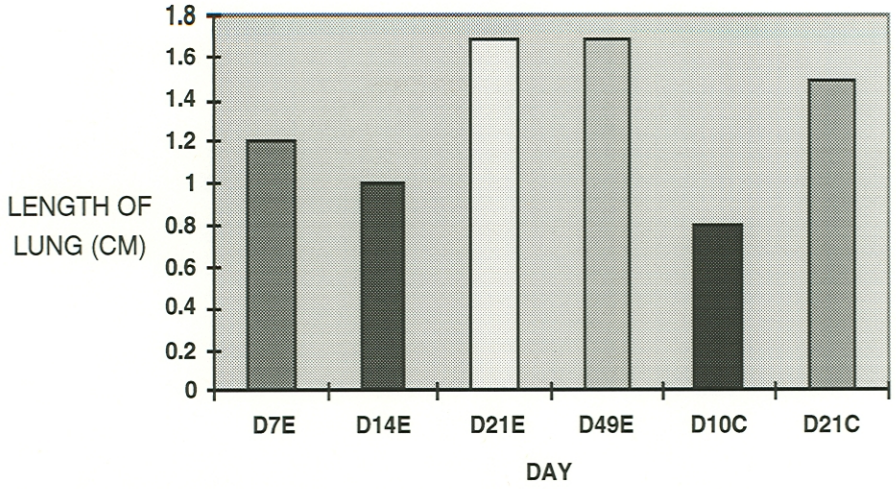

Figure 2: The elongation of the metamorphosing and control axolotl as a function of time.

sectional images were projected through a microscope onto a computer monitor, where the circumference of the lung could easily be measured. From the circumference of the lung, the area of the lung was determined and multiplied by its length (Figure 2) to give the volume. For each day of sacrifice, 15 cross-sections of each lung were observed and measured. These quantified sections included 5 from the anterior portion of the lung, 5 from the middle, and 5 from the posterior. These data were compiled and averaged together. The lung volume data found in Table 1 are also graphically seen in Figure 1. This figure allows for easy comparison between the experimental animals seen in the first four bars with the two control groups.

Surfacing counts, presumably to breathe, produced useful data for the comparison between the experimental animals and control animals as a function of time post-thyroxine injection. These data are seen in Figure 3. The experimental animals at days 14, 21 and 49 post thyroxine injection were out of the water 100% of the time, graphically represented as 50 times surfacing.

Photographs were also taken of the lung cross-sections at 100x magnification. They are included as Figures 4-9. These photographs were taken with the intention to show the lung wall, the vascularization of the lung, as well as the increase in surface area of the lung lumen as the animal underwent metamorphosis.

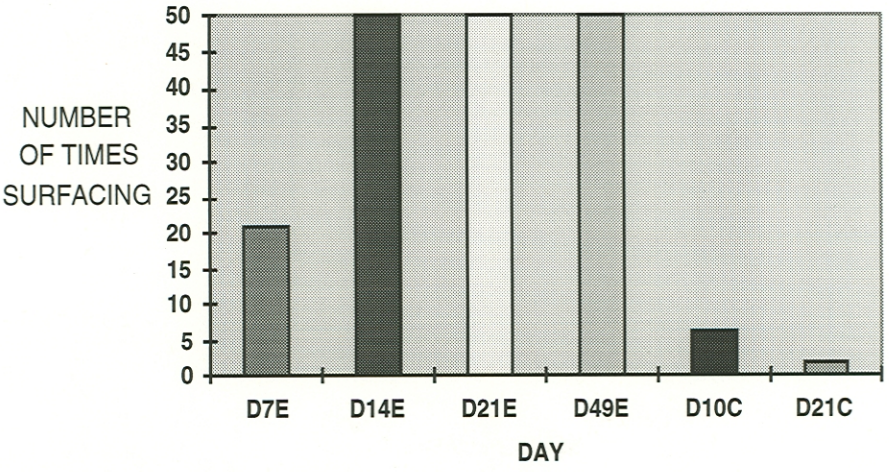

Figure 3: Number of times surfacing for air in a ten-minute period as a function of metamorphic time as compared to control animals.

Discussion

The axolotls were out of the water on rocks approximately 14 days after thyroxine injection, as seen in Figure 3. They were relying on their lungs for oxygen and carbon dioxide exchange, correlating with the absorption of their external gills. There was also a vast difference seen between the surface breathing patterns of the control and experimental animals as a result of thyroxine injection.

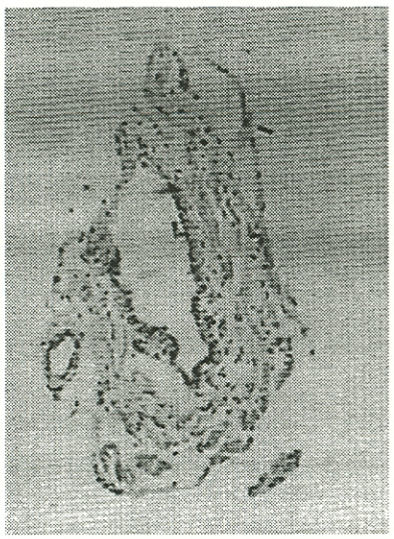

Figure 4: Cross-section of a metamorphosing axolotl lung 7 days post-thyroxine injection.

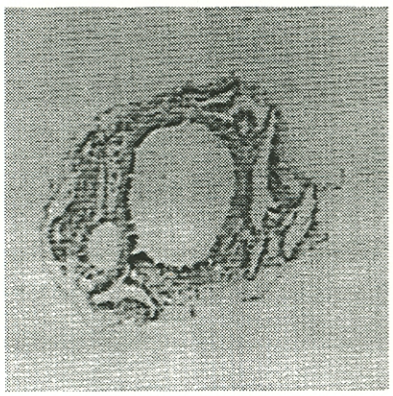

Figure 5: Cross-section of a control axolotl lung at 10 days post-saline injection.

As shown in Figure 1, the volume of the axolotls' lungs increased substantially between the 14th and 21st days, correlating with the absorption of the external gills and the animals' growing reliance on their lungs. There was also a noticeable difference between the D21E and D49E animals as compared to the D21C animals, suggesting these alterations are a result of thyroxine injection. The volume of the lung did not alter at the precise time that the gills were absorbed; perhaps they needed the external pressure from the animal forcing air into the lungs to signal lung alterations. Regardless, there was a large alteration in volume as a result of thyroxine injection

There was a decrease in lung volume between the 21st and 49th day (Figures 1, 7 and 9). When measurements were recorded, the external circumference of the lung was measured and not the internal surface area and volume of the lumen. Therefore, when the inner surface area increased on the 49th day due to invaginations of the external wall, my method of measurement did not account for this alteration. In all actuality, the gas exchange potential of the lung increased but the graphical representation shows a slight decrease in volume.

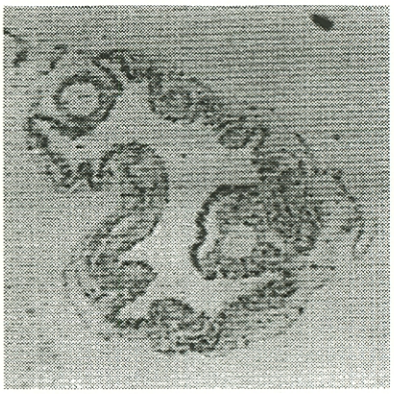

Figure 6: Cross-section of a metamorphosing axolotl lung 14 days post-thyroxine injecion

There was also a considerable decrease in the lung wall thickness. This is best seen by comparing Figure 4 to Figure 7. As seen in Figure 2, the length of the axolotls' lungs increased as a function of metamorphic time. The excess wall tissue seen in the earlier stages of metamorphosis possibly contributed tissue to the increase in lung length.

Using Figures 4-9 as a reference, there have been many noticeable anatomical alterations of the lung throughout metamorphosis. Figure 4 clearly shows a lung (7th day post-thyroxine injection) with a thick wall and small lumen. With the exception of one large, noticeable blood vessel, the lung was not well vascularized. This lung did not

offer an efficient mechanism for oxygen and carbon dioxide exchange.

Figure 5 (10th day post-thyroxine injection) shows a control animal at approximately the same stage of development as the 7-day experimental, but the control lung was not undergoing metamorphosis. There was also a small lumen, very little surface area, and limited vascularization. Therefore, this lung was similar to the day 7 post-thyroxine lung. These animals were also fully underwater, and their external gills were not noticeably decreased. Metamorphically speaking, they were in approximately the same stage. This was seen in their similar lung structures.

By the 14th day post-thyroxine injection (Figure 6), there was a major increase in lumen

Figure 7: Cross-section of a metamorphosing axolotl lung 21 days post-thyroxine injection.

surface area due to an increase in invaginations (compare to Figures 4 and 5). Many smaller blood vessels vascularize the lung. This lung was more efficient for oxygen and carbon dioxide exchange, correlating with the axolotls' obvious reduction in external gill size and increased external air breathing (Figure 2).

Figure 7 (21 days post-thyroxine injection) shows a massive anatomical alteration, correlated with an increase in lung volume, surface breathing, and lung length. The lung wall was greatly decreased, suggesting this tissue contributed to the growing length of the lung. This animal was completely out of the water on rocks and relied upon its lungs for gas exchange. This lung was vascularized (the upper right is a destroyed blood vessel) and had massive amounts of surface area, making gas exchange possible. However, this lung did have a large amount of dead, unproductive space in the lumen. The cross-section in Figure 7 (21st day post-thyroxine injection) can be compared to that of the day 21 control animal (Figure 8).

The cross-section comparable to the lung seen in Figure 7 is a control lung with a thick wall surrounding the lumen, very little surface area or vascularization, and little potential for

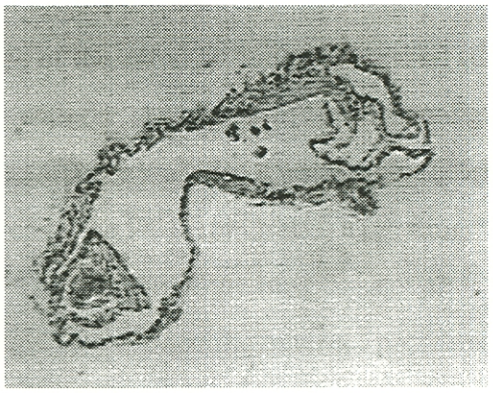

Figure 8: Cross-section of a control axolotl lung 21 days post-saline injection.

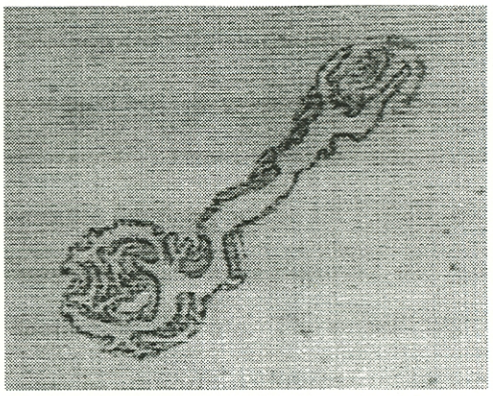

Figure 9: Cross-section of a metamorphosing axolotl lung 49 days post-thyroxine injection.

oxygen and carbon dioxide exchange. These differences can also be seen in the volumes and lengths of these lungs (Figures 1 and 2), suggesting a large anatomical alteration due to thyroxine injection.

Finally, Figure 9 shows a lung (collapsed) cross-section 49 days post-thyroxine injection. This lung was highly vascularized, and there was a cluster of vessels at either end of the lung as well as along the wall. The surface area of the lumen was greatly increased with many invaginations while the lung retained the large volume and length

seen in Figures 1 and 3. This lung was highly specialized for gas exchange.

Conclusions

Thyroxine-induced metamorphosis resulted in the absorption of the axolotls' external gills and a corresponding dependence on their lungs for gas exchange. The lung must undergo anatomical alterations to accommodate this demand. There was a thinning of the lung wall as well as an increase in length and lumen surface area. The increase in surface area was paralleled by an increase in lung volume as well as an increase in lung vascularization. These alterations were necessary to facilitate oxygen and carbon dioxide exchange in a post-metamorphic axolotl.

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