The Axolotl Newsletter

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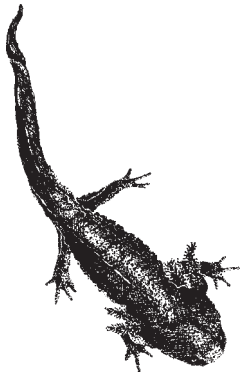
Back issues 1-25 of the Newsletter are available. Single issues cost $1.50. Unless individual numbers are requested, issues 1-9 and 11-14 are sent reduced in size and bound together. The set containing issues 1-9 costs $10, and the set containing issues 10-14 costs $5. Contents are listed on our website. Indices for numbers 1-15 are in issue 15 and for numbers 16-20 in issue 20. Issue 25 contains indices for issues 21-25.



Contents

Xochimilco Today

Note from the Director

We continue with our attempts to develop protocols for generating transgenic axolotls. Our strategy is to focus on each of the component steps which might comprise an ultimately successful procedure. Using the methods employed with *Xenopus* as a guide we have accomplished several of the steps. (See: http://www.welc.cam.ac.uk/~ea3/The.Amaya.Lab.Homepage.html)

First, sperm are collected by dissection of freshly collected vas deferens, and concentrated to an appropriate density (so that during subsequent microinjection each egg will receive 1-5 sperm). Second, sperm heads are swollen, so that when bathed in a solution of a foreign gene a suitable amount of gene will enter each sperm. This "swelling of sperm" is accomplished by adding 2.0-5.0 M NaCl to a sperm suspension. Within two hours at room temperature the sperm heads swell to several times their natural volume. Third, those swollen sperm are injected directly into freshly spawned, dejellied axolotl eggs, in order to determine whether they can drive cleavage. It is here that we are presently "stalled." Cleavage frequency varies dramatically from spawning to spawning.

Only approximately 10% of the injected eggs from some spawns cleave while from other spawns up to 25% cleave. Of those which cleave normally, only a fraction (approx. 25%) gastrulate, and only a few of those neurulate. We will work at treating the sperm in a less harsh fashion in an attempt to improve development of recipient eggs.

Once cleavage is achieved with a reliable frequency we plan to treat the swollen sperm with an appropriate foreign gene. Dr. Roy Tassava of Ohio State University is working closely with us on all aspects of this project and will provide a gene construct.

George M. Malacinski

News from the Axolotl Colony

Update on Fees

The Axolotl Colony has been charging for material for a bit over a year. We revised the fee schedule for the current season in order to make it fairer and easier to administer, while still trying to keep paperwork fairly simple. Prices for embryos are somewhat higher than last season, and there is no longer a cost ceiling for high volume embryo users. These charges help, of course, to defray some of the costs we incur in order to supply the material, but at this time they do not nearly cover all of the expenses involved in running the colony. The new price structure and invoicing system for embryos also reflects how different laboratories use embryos. Our embryo users fall mainly into two groups, those that use fewer than 1500 embryos and receive fewer than 10 shipments, and those that use greater than 3500 embryos and receive 20 or more shipments over the course of the season. The former will now be invoiced monthly, while high-volume users with standing orders will be invoiced quarterly. We hope this system will keep paperwork manageable.

We would like to thank all of you who have used our services during this period of adjustment for your patience and understanding as we learn and improve.

Also, please note: All users of Axolotl Colony material are required to register with the colony (one registration per lab), but you do not need to register more than once. Please do, however, let us know if your address, telephone, e-mail, etc. change. If your institution requires a purchase order, please have the number available when you make your order.

Newsletter Going On-Line

Beginning with this issue, the Axolotl Newsletter will now be available on-line. To access the newsletter, just go to the Axolotl Colony website (http://www.indiana.edu/~axolotl) and choose The Axolotl Newsletter from the main menu. The on-line issue will be substantially the same as the paper copy.

We encourage all of you with access to the World Wide Web to read future issues of the Newsletter on the web. Anyone who would prefer to no longer receive the paper copy should send me an e-mail message to that effect (duhon@indiana.edu). I will notify all of

you by e-mail whenever a new issue appears on the web.

The Axolotl in Mexico

Sandi and I had the opportunity to go to Mexico in January, 1997 and work with Virginia Graue of the Autonomous Municipal University at Xochimilco (UAM-X). She is Director of CIBAC, which is a center for the study of the native aquatic fauna (axolotls, frogs, fish, etc.) of Xochimilco and part of a larger Ecological Restoration project for Xochimilco. While there we were able to see the axolotl in its native habitat as it is today, and we were able to assist Virginia in her efforts to establish a breeding colony of axolotls at CIBAC, which is located on the banks of the Canal de Cuemanco, one of Xochimilco's major canals. Virginia's work in Mexico is vital if this endangered species is to survive in its natural habitat.

Susan T. Duhon

Indiana University Axolotl Colony

Price List

current Fall 1997

Embryos

One-time or occasional (<1500 embryos annually)	Multiple (>1500 embryos annually, frequent shipments
$30 per 100 embryos or fraction thereof, billed monthly	$150 per 500 embryos or fraction thereof, billed at the end of each "quarter" (Oct-Dec; Jan-Mar; Apr-Jul)

Darkly pigmented embryos are sorted at blastula.
Albino embryos are NOT sorted, viability varies.
Mutant spawns may be priced higher. Please inquire about prices and availability of specific mutants.

Larvae, Juveniles, and Non-breeding Adults

Priced per animal.

Size Range**	Cost per Animal ($)***	Quantity Prices
Hatchlings	0.25	$25 per 100
2-3 cm larvae	0.50	$25 per 50
3-5 cm larvae	1.00	$25 per 25
5-8 cm juveniles	5.00	
8-15 cm juveniles/sub-adults	10.00	
Healthy cull adults (non-breeding)	15.00	

**All sizes may not be available. Call or e-mail to find out current availability. In some cases when space allows, animals can be raised to fill orders placed in advance.

*** Billed monthly.

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-

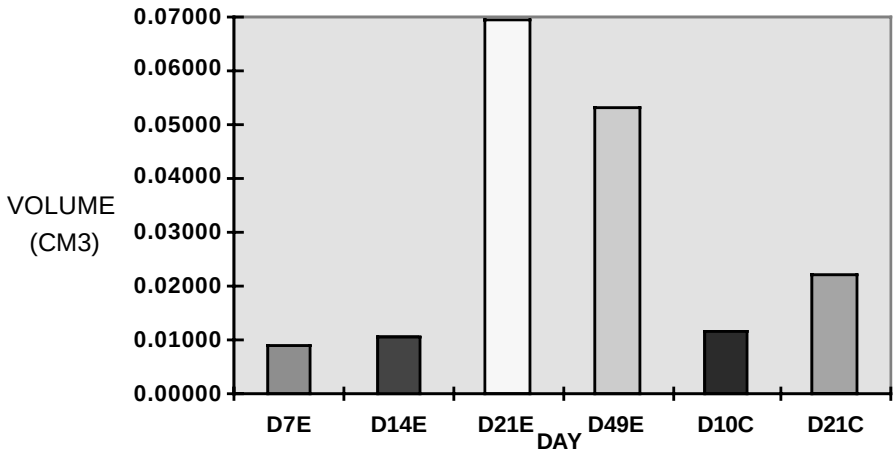

Figure 1: Graphical representation of lung volume as a function of time.

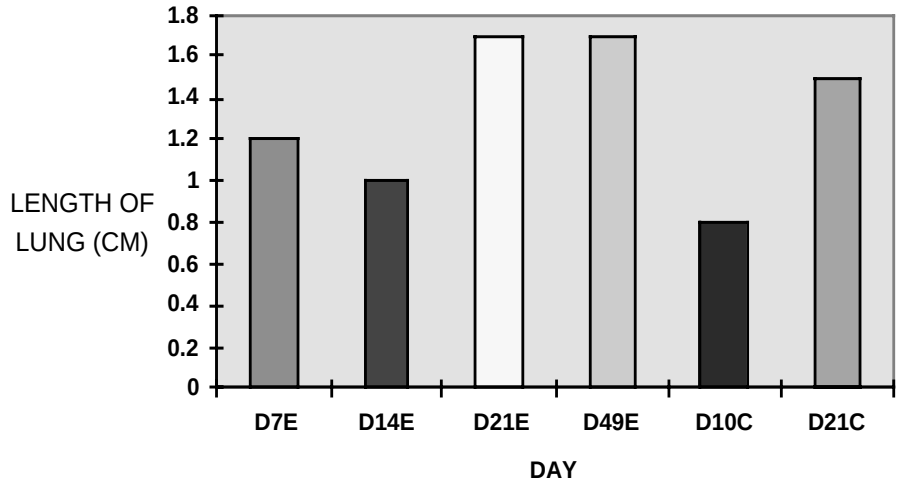

Figure 2: The elongation of the metamorphosing and control axolotl as a function of time.

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.

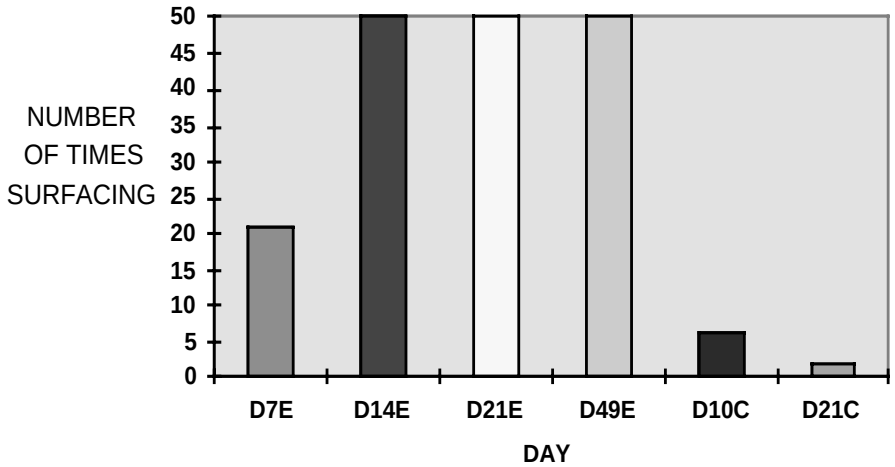

Figure 3: Number of times surfacing for air in a ten-minute period as a function of metamorphic time as compared to control animals.

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.

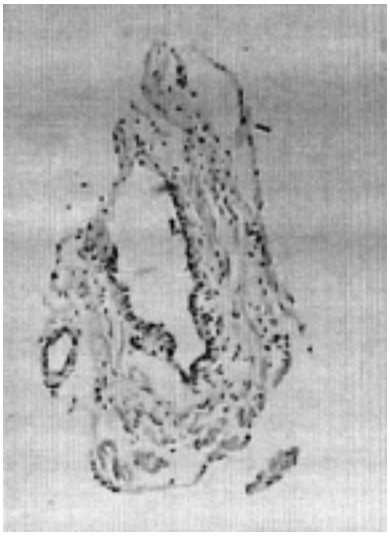

Figure 4: Cross-section of a metamorphosing axolotl lung 7 days post-thyroxine injection.

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.

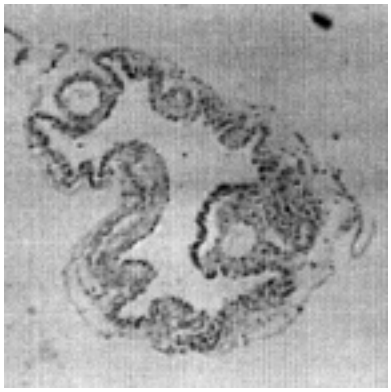

Figure 6: Cross-section of a metamorphosing axolotl lung 14 days post-thyroxine injecion

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

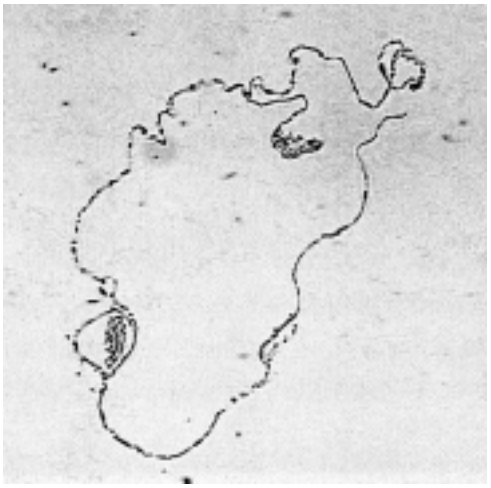

Figure 7: Cross-section of a metamorphosing axolotl lung 21 days post-thyroxine injection.

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

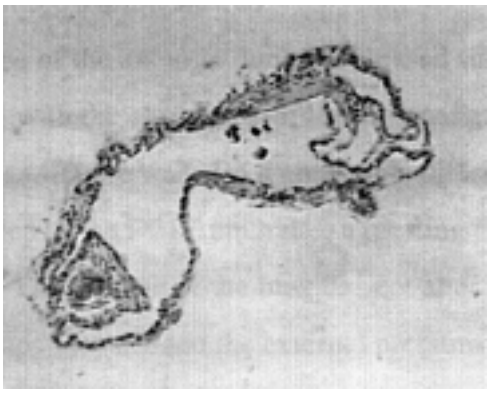

Figure 8: Cross-section of a control axolotl lung 21 days post-saline injection.

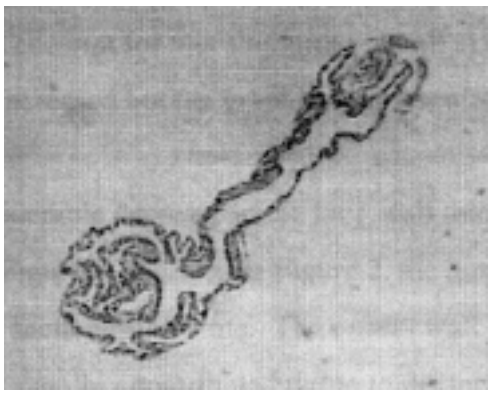

Figure 9: Cross-section of a metamorphosing axolotl lung 49 days post-thyroxine injection.

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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Effect of 60 Hz Ambient Magnetic Fields on the Development of Axolotl Embryos

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Abstract

This work documents investigations into the possible teratogenic effect of extra low frequency magnetic fields on the development of axolotl (*Ambystoma mexicanum*) embryos. The investigation was done by exposing a group embryos to power frequency magnetic fields and comparing the number of healthy developed ones to those of a control group.

The results indicate that ambient and above ambient levels of low frequency magnetic fields have no adverse effect on the development of the axolotl embryos as measured by the parameters used.

Background on Ambient Magnetic Fields

The naturally occurring magnetic field of the Earth is essentially static. Its vertical component has a magnetic flux density that averages about 50 microTesla (μT) or 500 milliGauss (mG) at middle latitudes. The field peaks at the magnetic poles to about 67 μT, and has a value of zero at the magnetic equator. The horizontal component is 33 μT at the magnetic equator and zero at the magnetic poles.

The peak value of magnetic flux density underneath a double circuit, 500 kiloVolts (kV) transmission line carrying a total of 5000 MegaWatts (MW) is less than 3.5 μT. This value drops to about 0.5 μT at the edge of the right-of-way.

Hypothesis

Review of literature (Chacon et al., 1990; Martin, 1992; Nagai and Ota, 1994; Pafkova and Jerabek, 1994; Santini, et al., 1994; Veicsteinas et al., 1996) indicates that adverse effects of electromagnetic fields appear to be caused by high levels of field strength or high frequencies.

It is expected that ambient levels of 60 Hz magnetic fields (given in Table 1), should have no adverse morphological or behavioral effects

Appliance	Magnetic Flux Density, μT		
	3 cm	30 cm	1m
Can openers	1000-2000	3.5-30	0.07-1
Hair dryers	2-2000	0.01-7	< 0.01-0.3
Electric shavers	15-1500	0.08-9	< 0.01-0.3
Drills	400-800	2-3.5	0.08-0.2
Mixers	60-700	0.6-10	0.02-0.25
Portable heaters	10-180	0.15-5	0.01-0.25
Blenders	25-130	0.6-2	0.03-0.12
Television	2.5-50	0.04-2	0.01-0.15
Irons	8-30	0.12-0.3	0.01-0.025
Coffee makers	1.8-25	0.08-0.15	< 0.01
Refrigerator	0.7-0.7	0.01-0.25	< 0.01

Table 1. Magnetic field level at 60 Hz near various home appliances in a typical U.S. home (Electric Power Research Institute, 1989).

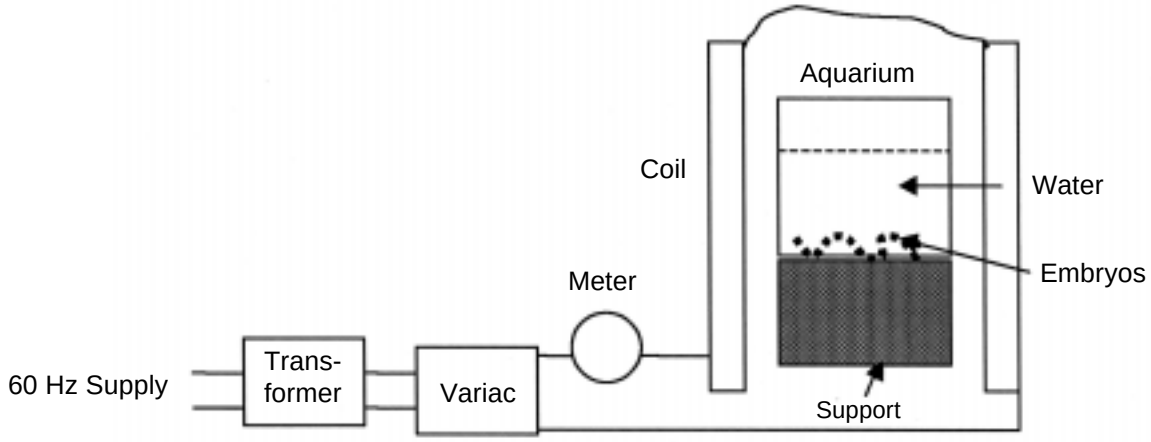

Figure 1. Experiment Setup

on the developing axolotl embryos. The following set of experiments were design to test the validity of this hypothesis.

Materials and Methods

The experiments involved subjecting a group of fertilized eggs to a 60 Hz electromagnetic field from the early stages of development until they hatched. Various field strengths were used from slightly below to as much as triple the ambient values. Various orientations of the exposed eggs with respect to the direction of the field were observed. The hatched embryos were scored and compared to a control group for mortality rate and morphological abnormality.

Experiment Setup

The present work (Abdel-Hadi, 1997) comprises a number of experiments in which axolotl embryos were subjected to 60 Hz electromagnetic fields. The embryos were placed in two identical glass aquaria, sealed with non-conducting aquarium silicone: one was exposed to fields generated by a Helmholtz coil, the other was shielded by placing it inside a box-like metal mesh and grounded through a connection to the laboratory's water pipes.

A Helmholtz coil is made up of a pair of identical coils, placed parallel to each other. When the distance between the coils is equal to their radius, the magnetic field in the space between them is essentially uniform. The magnetic flux density between the coils is a function of their diameters, number of turns and the current flowing through them.

The setup, including the circuit connection is shown in Figure 1. Figure 2 shows the aquarium, the pair of Helmholtz coils and the control aquarium.

Figure 2. The Aquaria and Helmholtz Coil

Experiments and results

The experiment was repeated four times between February 1994 and May 1996. The following are the particulars of each experiment and the results. In all of the experiments, the embryos were in stages of development numbers 4 to 7 during exposure.

Experiment 1. Each aquarium contained fifty-five eggs. The exposed group was subjected to a 1500 mG magnetic field. Only one egg in each aquarium did not develop. It was noticed that before hatching, all the embryos had a curl shape and were oriented in a vertical plane.

Experiment 2. Forty-eight embryos were placed in each aquarium. The field strength was 15 μT. Six embryos did not develop in the exposed aquarium and four of the control ones failed to develop. The orientation of the embryos was random.

Experiment 3. Sixty-two embryos were placed in each aquarium. Again the field strength was set at 15 µT. Twelve embryos failed to develop in the exposed aquarium and thirteen in the control aquaruium. The orientation was random.

Experiment 4. Seventy-three embryos were placed in each aquarium. The field strength was doubled to 30 µT. Five of the control group and seven of the exposed group did not develop. The orientation was again random.

Table 2 summarizes the results of the six experiments.

In all of the above experiments, the embryos that developed were free from morphological abnormalities. In order to test behavior, the embryos were fed brine shrimp after hatching, and there was no evidence of any behavioral abnormalities, since they showed orange abdomens. The variability in the number of developed embryos could be due to the parents being different.

The 'p values' for all of the above experiments, as measured by the chi-square test, is greater than 0.05 and hence the difference between the control and exposed embryos is not significant.

Experiment	Field Strength mGauss	Number Of Embryos						P value
		Control			Exposed			
		in group	developed	%	in group	developed	%	
1	1500	55	54	98	55	54	98	0.5173
2	1500	48	44	92	48	42	88	0.7379
3	1500	62	49	79	62	50	81	0.8177
4	3000	73	68	93	73	66	90	0.7611
Total		238	215	90	238	212	89	

Table 2. Summary of Experimental Results

Conclusions

This work investigated the possible effects of ambient and above ambient levels of North America power frequency magnetic fields on the axolotl embryos. The results of the study clearly indicate that exposure of axolotl embryos to 1500 and 3000 mG, 60 Hz sinusoidal magnetic field; from the early cleavage stages until hatching (9 to 10 days), do not adversely affect the developing embryo. The scoring was based on:

1. Failure to develop.
2. Morphological abnormalities.
3. Post hatching abnormal behavior.

The fact that a relatively large number of the embryos were aligned with the field in experiment #3 cannot be attributed to the magnetic field since these results could not be reproduced.

Most of the adverse effects reported in the literature resulted from exposing embryos to high frequency, or to low frequency pulsed magnetic fields containing high frequency components. This, together with the results of the present study, indicates the need to establish a relationship between possible adverse effects on organisms and the field frequency. Pure fundamental power frequency sinusoidal waves that are free of harmonic contents appeared to have no effect on axolotl embryos. These results are in agreement with those reported by other researchers such as Chacon et al. (1990), Martin (1992), Santini et al. (1994) , Pafkova and Jerabek (1994), Nagai et al. (1994) and Veicsteinas et al. (1996).

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The Axolotl and its Native Habitat—Yesterday and Today

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The Mexican axolotl (*Ambystoma mexicanum*) has been an important laboratory animal and model system for more that a century. In spite of this long history in research, however, we know relatively little about the axolotl's natural history. And, in spite of the many axolotls that have been bred and raised in laboratory colonies, we know practically nothing about its natural habitat.

Our laboratory species of axolotl* came originally from two lakes, Xochimilco and Chalco, in the Valley of Mexico. Today, however, the Valley of Mexico is home to an estimated 18 million people. As a result of the growth of Mexico City, in this century especially, these lakes have been reduced from an area of perhaps 120 square kilometers at the beginning of the sixteenth century, to the few hundred hectares of canals and lagoons of modern Xochimilco. In fact, these canals only exist at all because they are recharged with treated sewage effluent. Figure 1 shows a sign at a site where treated effluent is pumped into Xochimilco.

Thus the natural habitat of the axolotl cannot merely be studied, it must be reconstructed, as best we can, from historical documents and from archaeological and geological studies.

The Valley of Mexico is actually a closed basin formed during the tertiary without natural drainage. In prehispanic times the lower portions of the basin were occupied by a system of five lakes. Overflow from the higher lakes, Chalco and Xochimilco in the south and Zumpango and Xaltocan in the north, flowed into Texcoco, the lowest lake in the center of the basin. Texcoco, lacking any outlet except evapotranspiration, was extremely saline (Figure 2).

Figure 1. Sign at a site where treated effluent is pumped into Xochimilco.

Lakes Chalco and Xochimilco were situated in the southern basin at an altitude about three meters higher than Lake Texcoco and separated from it by a low range of hills known as the Sierra de Santa Catalina. In addition to precipitation and surface runoff, they were fed by deep perennial springs located near the southern boundary of Xochimilco. Although traditionally they are considered to have been two lakes, they really comprised a continuous body of freshwater lagoons and

Figure 2. Basin of Mexico around 1519.

* The species of neotenic salamander that we maintain in the Axolotl Colony and that is a common research animal is *Ambystoma mexicanum*, originally native to lakes Chalco and Xochimilco. Although we refer to this species as the Mexican axolotl, in reality various other lakes in central Mexico are or were home to a number of species of non-transforming Ambystomatid salamanders. See Shaffer, H.B.1989. Natural history, ecology, and evolution of the Mexican "axolotls." Axolotl Newsletter **18**:5-11.

swampland in a shallow saucer, the rim of which was marked by the 2240 meter contour of elevation. The actual water level would have varied with periods of drought and flood, but apparently seldom rose above the 2240 contour (Figure 3).

Ambystoma bones recovered in archaeological excavations of lakeshore sites in the Xochimilco–Chalco basin show that from at least 6000 B.C. axolotls were part of a rich freshwater lacustrine habitat. They shared this habitat with lake turtles, waterfowl (including geese, ducks, coots, and grebes), freshwater mollusks and ostracods, and fish (*Chirostoma*, *Girodinichthys*, and Cyprinids). The shorelines supported *Cyperaceae*, *Sparganiaceae*, rushes, and cattails. Submerged and floating aquatic plants included water lentils, horsetail, *Myriophyllum*, and *Epilobium*. After 3000 B.C., willows, *Malvaceae*, *Liliaceae*, and *Umbelliferae* became more frequent (Niederberger, 1979).

People began modifying the natural habitat of the axolotl by about 1000 A.D., when the indigenous people of the region are thought to have begun using a system of horticulture and irrigation known as *chinampas*. Chinampas were long, narrow fields created in swampy areas by heaping up muck and aquatic vegetation dredged up from the swamp. Willow stakes were driven into the bottoms around the edges and joined by wattles to enclose and contain the mud. Each chinampa was bordered on at least three sides by water, and was thus irrigated by seepage from the surrounding canals. The chinampas were very fertile and productive, and they yielded as many as four crops in a year (Rojas Rabiela, 1991).

By the mid-thirteenth century the Xochimilco-Chalco region had been incorporated into the Aztec state centered at Tenochtitlan. The Aztec capital was located on an island near the western shore of Lake Texcoco. The Aztecs constructed an elaborate system of causeways, dikes, and canals which, in conjunction with the chinampas, controlled flooding and separated fresh and saline waters. In particular, the Aztecs built a long dike that divided Texcoco into two parts and kept the saline waters of eastern Texcoco from the western section around Tenochtitlan,

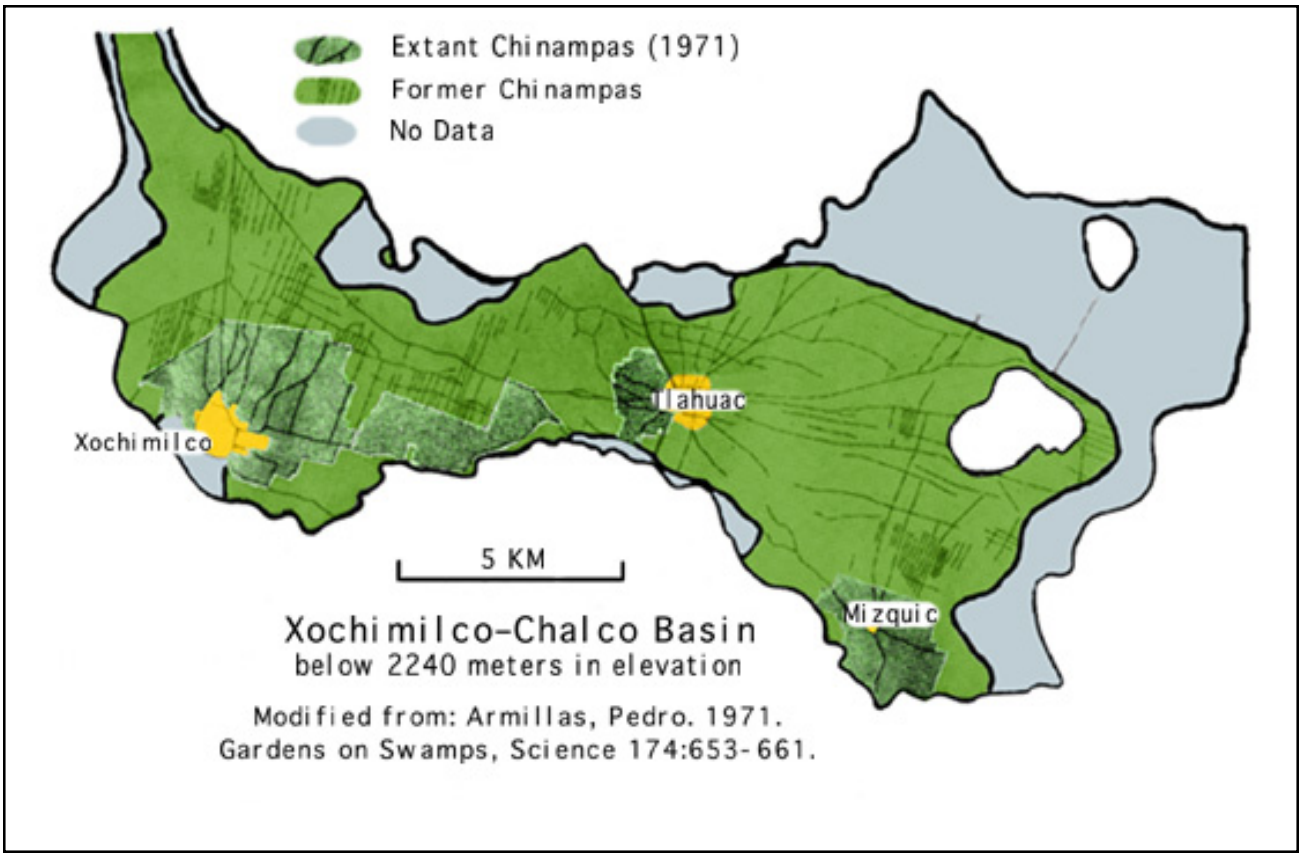

Figure 3. The southern basin.

which they called the Laguna de Mexico. In addition, a causeway or dike crossing from north to south through Tlahuac separated Xochimilco from Chalco (Sanders, et al., 1979).

During the Aztec period nearly the entire Xochimilco-Chalco basin was devoted to chinampas horticulture, as was the Laguna de Mexico around Tenochtitlan. Also, as a result of the Aztec waterworks, saline water was largely prevented from entering the southern lakes, even during periods of flood (Armillas, 1971).

Thus the habitat of the axolotl was significantly affected during the period of the Aztec empire in at least two ways: first, by the spread of the chinampas, which involved the construction of ditches and islands, and second, by the construction of waterworks which controlled water levels and prevented the incursion of salt water into the axolotl habitat. Its habitat may have been enlarged as well by the expansion of fresh water horticulture into the district around Tenochtitlan. The impact that these historical changes had on the species can only be speculated upon.

The chinampas system and the Aztec waterworks were at their peak when the Spanish Conquistadors arrived in 1519. What ensued was two years of warfare during which the waterworks were largely destroyed. As a result, during the sixteenth century, the southern basin experienced both flood and drought as the Spanish worked to protect the nascent Mexico City, located on the site of Aztec Tenochtitlan, from flooding. During this and subsequent periods, many of the Aztec waterworks were rebuilt, and efforts were also begun to drain the region about the city (Palerm, 1990).

Even though in time the Laguna de Mexico was drained by the construction of a canal for that purpose north from Texcoco, lakes Xochimilco and Chalco remained largely intact throughout the seventeenth, eighteenth, and nineteenth centuries. They continued to be recharged by surface runoff and by perennial springs. Moreover, throughout this period an outlet was maintained via the Canal de la Viga, which carried water and commerce from Lake Xochimilco to Mexico City, even after the original outlet to Texcoco was lost due to basin drainage. Chinampas agriculture continued to be practiced but began a long period of decline (Rojas Rabiela, 1991).

The axolotl was well-known to the Aztecs and other indigenous peoples in the area, who included it as part of their varied diet, and several early Spanish accounts describe it. For instance, Sahagún wrote in his monumental work on the indigenous people of Mexico (Sahagún, 1938):

> **There are some creatures in the water that are called axolotl that have feet and hands like small lizards, and they have the tail of an eel and the body as well; they have a very wide mouth and whiskers at the neck. It is very good to eat; it is the food of lords.***

Francisco Hernández also described the axolotl in his treatise on Mexican natural history. Although this work was not published until about 1648, it was based on material that he collected during his travels in central Mexico between 1570 and 1577. Other notable mentions of the axolotl include the account by Shaw in his Naturalist's Miscellany in 1798 (where it was designated *Gyrinus mexicanus*) and the description published in 1811 by Georges Cuvier, based upon preserved specimens (See Smith and Smith, 1971).

There are a few accounts which give us a glimpse of the axolotl and its habitat during the nineteenth and early twentieth century. José Velasco published a paper in 1880, "Anotaciones y observaciones al trabajo del Sr. D.A. Weismann sobre la tranformación del ajolote mexicano en *Amblistoma*" in La Naturaleza 5:58-84. This article has been translated into English (Dranz et al, 1971). Velasco was interested especially in neoteny and metamorphosis as he found it in the various salamander populations of the basin, but he affords us some interesting glimpses of the axolotl in its native habitat. He tells us, for instance, that at that time, lakes Chalco and Xochimilco were permanent lakes and the water was of very good quality. They were largely covered by floating vegetation and many plants also grew on the bottom. The axolotls were usually found at a depth of about one and a half meters or less, and, during the night only, near the surface. They were not to be found in the "flooded lands" or in canals, trenches, or wells.

Velasco also reported color phases among

* Hay unos animalejos en el agua que se llaman *axólotl*, (que) tienen pies y manos como largartillos, y tienen la cola como anguila, y el cuerpo también; tienen muy ancha la boca y barbas en el pescuezo. Es muy bueno de comer; es comida de señores.

the axolotls in the lakes. He described "yellow-spotted" individuals in Lake Chalco that lived in "salty water." He also mentions two types in Lake Xochimilco, one darker than the other. According to Velasco the water in the southern lakes was "not completely uniform throughout, in some localities being lightly salty and in others fresh. Each type [of axolotl] is limited precisely to a certain type of water: the darkest ones live in that which contains the least salt and the yellow-spotted ones in that which is more salty."

This description can be supplemented by that of H. Gadow, writing in 1903 . At this time lakes Chalco and Xochimilco were still fed by deep springs, "thirty or forty [9 to 12 meters] and more feet in depth." About half the surface was filled with chinampas. The depth of the lake averaged around "five to ten feet [1.5 to 3 meters], shallower to the northwest," where it was swampy. Water near the springs was clear, but muddier and darker the farther from the springs one moved. It was full of decomposing vegetable matter, fish, insect larvae, *Daphniae*, worms, and axolotls. The Canal de la Viga continued to be an important artery for the transport of the produce of the chinampas to Mexico City.

Gadow reported that the axolotls bred at the beginning of February, fastening the eggs to the water plants. By June "they were all grown into big, fat creatures ready for the market." Later in the summer, according to Gadow, "they take to the rushes, in the autumn they become scarce." These axolotls were dark in color "never piebald or marbled with yellow."

Velasco claimed that at least some axolotls from Xochimilco and Chalco transformed into a terrestrial form. Gadow, however, asserted that axolotls in these lakes remained entirely aquatic.

Urban growth eventually took its toll on lakes Xochimilco and Chalco, although the impact was apparently not large until the twentieth century.

In 1846 well drilling began in the basin after it was discovered that potable groundwater was available under artesian pressure. By the early twentieth century, drainage works were underway in the southern basin. As Mexico City continued to grow, so did the need for water, and Xochimilco's springs were tapped by an aqueduct during the 1920s. The springs dried up entirely during the 1930s. In 1953 well drilling accelerated in the southern basin, when many wells in the central basin were closed because of severe subsidence as sediments compacted after ground water removal. As a result of all these changes, today Lake Chalco is gone and only a small remnant of Lake Xochimilco remains (National Research Council, 1995).

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Instructions for Contributors

Contributions pertaining to axolotls or other urodeles or more generally to amphibians are welcome. All of the following categories are encouraged:

short reviews
research notes
technical comments
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announcements
inquiries for information
colony descriptions or directories
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Authors are encouraged to submit line drawings, diagrams, or black-and-white photographs to accompany the written contribution.

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