

# The Effect of Ibuprofen on Tail Regeneration in Axolotls (*Ambystoma mexicanum*)

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## Overview

**Purpose:** To determine the role of inflammation and nerves in the regeneration process to develop a model for increasing regeneration in humans.

**Experimental Hypothesis:** If ibuprofen was administered during the entire regeneration process of the tail of embryonic axolotls at stage 42<sup>2</sup>, then tail regeneration would be inhibited because inflammation and the nerves that send the message of damage to the brain would be inhibited.

**Null Hypothesis:** There would be no statistically significant difference between the means of the control and experimental group.

**Independent Variable:** The presence and concentration of ibuprofen in the rearing water (Figure 3).

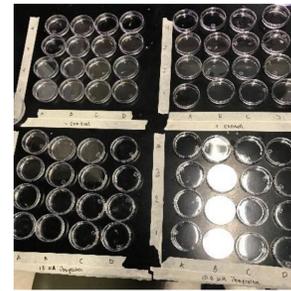
**Dependent Variable:** The amount of regeneration measured by the length of the axolotl from head to tail tip (Figure 1).

## Introduction

As humans age, regenerative abilities decline. The body continues to degenerate and cannot regenerate to compensate, increasing the risk of developing diseases or disorders. Determining means to increase regenerative abilities in humans is necessary to diminish the process of aging and decrease the risk of disease<sup>3</sup>. Studying regenerative organisms, such as the axolotl, could lead to the discovery of important pathways involved in the regeneration process<sup>1</sup>. The effect of ibuprofen was tested due to its anti-inflammatory functions. The tails of axolotl embryos were amputated 2 mm from the distal end of the tail immediately after hatching. The embryos were placed in 8 mL of either control solution, 1.0 μM ibuprofen, or 10.0 μM ibuprofen, with each group containing 16 embryos. The embryos of the negative control group did not have amputated tails. The length of the embryos from top of the head to the tail tip was measured every 24 hours for 72 hours post amputation. The results indicated that ibuprofen inhibits regeneration in axolotls (ANOVA  $p = 0.00144$ ). It was then concluded that the inflammatory and nerve pathways inhibited by ibuprofen are involved in the regeneration process. Future research should focus on the role of inflammation in human regeneration.

## Materials & Methods

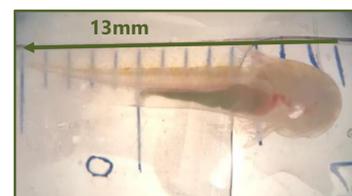
Blastula stage axolotl embryos were sorted and organized into individual wells containing artificial pond water.



The embryo's tails were amputated at 2mm from the distal end of the tail. The embryos were placed in the appropriate rearing solutions.



Over 72 hours post-amputation the embryos were placed in a bath of benzocaine anesthesia every 24 hours to be viewed and measured under a microscope.



At 72 hours post-hatching the embryos were sacrificed.

Figure 1

## Results

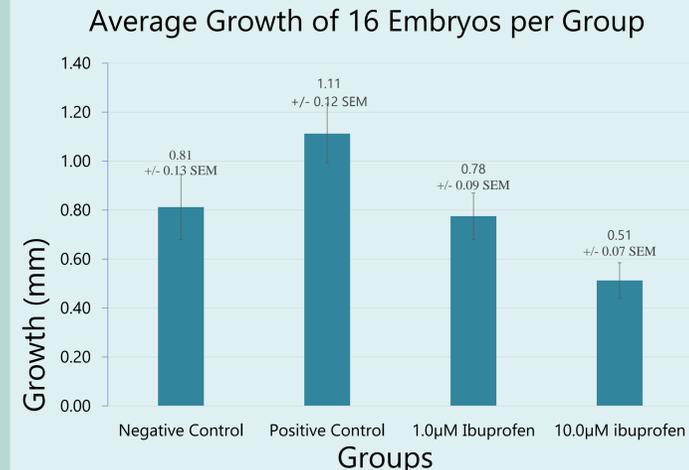


Figure 2

## Statistical Analyses

TABLE 1

ANOVA						
Source of Variation	SS	df	MS	F	p-value	Fcrit
Between Groups	2.90	3.00	0.97	5.84	0.00144	2.76
Within Groups	9.92	60.00	0.17			
Total	12.82	63.00				

TABLE 2

t-tests results			
Group 1	Group 2	Variance	p-value
Negative Control	Positive Control	Equal	0.03
Negative Control	1.0μM ibuprofen	Equal	0.40
Negative Control	10.0μM ibuprofen	Equal	0.04
1.0μM ibuprofen	Positive Control	Equal	0.01
10.0μM ibuprofen	Positive Control	Equal	0.00
10.0μM ibuprofen	1.0μM ibuprofen	Equal	0.03

## Applications to Biotechnology

- Development of pharmaceuticals to improve regenerative abilities as humans age to prevent age-associated diseases.
- Development of pharmaceuticals that target cancer cells to decrease regeneration and growth of cancer.

## Conclusion

The statistical analysis ( $p < 0.05$ ) allowed the null hypothesis to be rejected (TABLE 1, TABLE 2). Likewise, results (figure 2) support the experimental hypothesis. It can be concluded that ibuprofen is involved in regeneration in axolotls. This study reinforces and affirms the relationship of both the nerve and the inflammatory response in the process of regeneration.

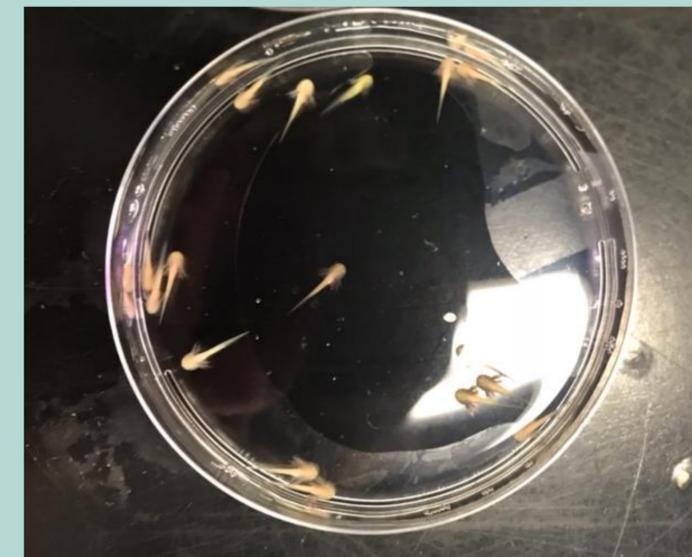


Figure 3

## Acknowledgements

- Professor Stephen Randal Voss, Ph.D., University of Kentucky

## References

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- See additional attached references