Histological Techniques Involved in Studying Salamander Skin

Lillie Arps, Kevin Gribbins
University of Indianapolis, Biology Department, IN 46227

ABSTRACT

The skills of embedding, sectioning, and staining techniques provide qualitative data for the histological study of the skin within salamanders. The salamander used in this study is the Cave Salamander, Eurycea lucifuga. There are no current studies on the histology of the integument that also provide information on the structure of their mucus and granular glands in this salamander. Through preliminary micrograph collection, we can see that both basic and acid stains provide insight on the architectural organization of the dermis with its different glands, and the muscular tissue underneath. To gather the high-resolution micrographs provided in this study, one must first master embedding skin tissue within plastic polymers. The samples must then be sectioned by a microtome and placed on a slide for visualization with high and low pH stains. This routine and following proper protocol for histological research are very technical and require a lot of practice and time to master. During the last year, I have worked on learning these techniques to gather quality histological data in cave salamander skin. The glands and general anatomical structure of the cave salamander skin is like that of the plethodontid species studied to date. The glands show similar stain properties to that of other species within the Plethodon genus. The mixed glands seem to be smaller in cave salamanders and most likely apocrine in their secretion type. We would like to verify this secretion pattern with TEM and also look at the subcellular details of the glandular cells and the epidermal layers in a continuation of this project within the honors college.

INTRODUCTION

Eurycea lucifuga, better known as the cave salamander are found throughout the eastern United States. They range from a yellow, orange to a reddish color with possible black or dark brown spots. Being apart of the the plethodontid clade, they are lungless amphibians that breathe through their skin.

There has been very little research on the histology of the *Eurycea lucifuga*. Within the Plethodontidae family, cave salamanders shows similar histological characteristics with that of it's closest relatives. However, there is very little information on the secretion patterns as well as their granular cells and epidermal layers of the skin within this species.

During the past year I have been practicing proper histological staining protocol to continue researching the histology of the cave salamander skin. The steps and skills required are very tedious to learn and take a lot of time and practice, which I've worked on for the past year. Through that time I have finally mastered these techniques to finally create my own data on the topic in spite of a demanding schedule of my course work and being a student athlete. I hope to continue this research in the upcoming years as part of my honors project.

MATERIALS and METHODS

Following the proper Histological Procedure for Staining and observing tissues require three main steps of embedding, sectioning and staining the tissues.

For the embedding procedure:

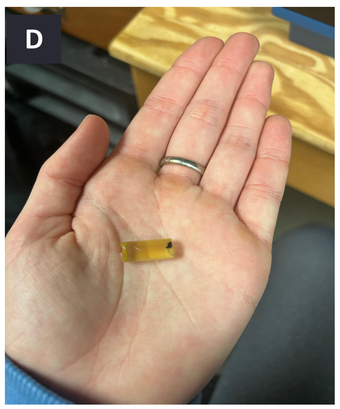
- the dermal layers are removed and washed with cacodylate for 10 minutes with the process repeated 3x
- The samples are fixed with 2% osmium tetroxide for 2 hours
- They are rinsed with deionized water for 15 minutes and this step is repeated 2x
- The samples are then dehydrated in an increasing grade of ethanol solutions
- Samples are covered with 1:1 of propylene oxide then 1:2 solution of propylene oxide
- Lastly, they are incubated in plastic epoxy: 10g ERL 4221, 8g DER 736, and 25g NSA
- The samples are incubated for 2 hrs and then cured in a iso temperature vacuum oven for 48 hrs

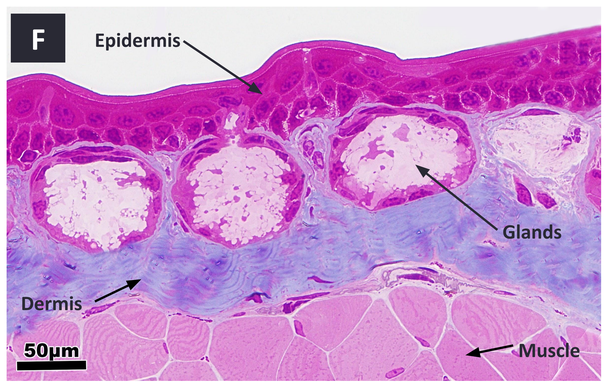
For the sectioning procedure:

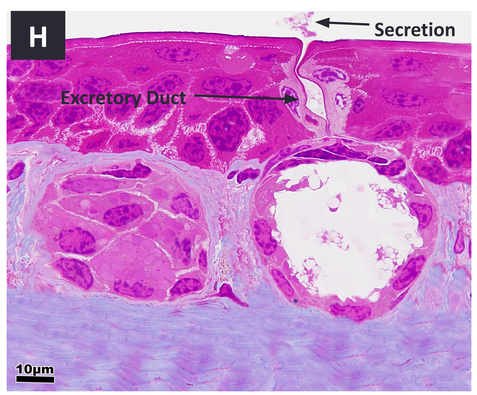
- The samples are placed within the microtone to section
- A glass blade is inserted to cut the sample. It is important to use a sharp blade to get clean cuts of the sample
- The angle of the sample or glass cutter is adjusted to ensure a full cut of the section
- The glass cutter is then moved up or backwards to cut the sample
- After a piece is sectioned, the thin cut sample is placed onto a clean slide

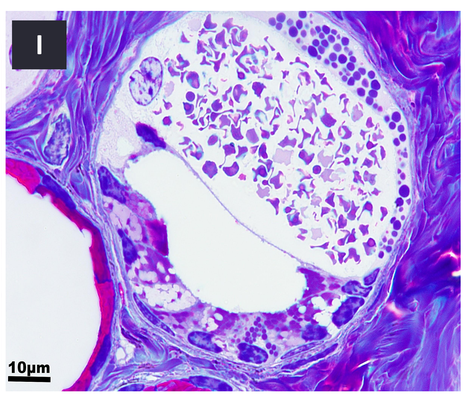
Tissue Staining:

- The slide with the sample is placed onto a hot plate to allow the tissues to adhere
- The sample is then stained with basic fuchsin and then rinsed with deionized water
- The sample is then stained with toluidine blue and rinsed with deionized water
- After these steps, the slide can be placed onto the microscope to observe the histological structure and pictures can be taken with an attached digital camera.









RESULTS & DISCUSSION

Microtone (Figure A and B)

Over the past year I have worked the most with this machine to learn specific techniques on creating quality samples for data collection. For each sample, the the microtone must be adjusted for the glass blade (shown in figure B) and the sample is lined up perfectly to get clean cuts from the blocks. This is a very tedious process or skill to master as going to far forward could damage the sample or the blade and being too far back will not section the sample. As well, you need to adjust for any possible angle presented on the sample surface to get a full cut of the entire face of the sample. With sectioning the samples into such thin and small pieces, there is little room for error and is why it took me a year to learn to work with the machine is a consistent manner.

Microscope (Figure C)

With the prepared slides, the microscope is used to observe and collect our digital data. The Olympus light microscope has four different magnification used for broad or magnified pictures of the tissues. There is a computer attached to the microscope that can be used to easily critique and observe characteristics of each slide produced.

Histological Structure (Figures F, G, H, and I)

The pictures exemplify the histological structure of the cave salamander. These images were cut and gathered by Dr. Gribbins with a diamond knife from thin sections cut from my faced blocks. The dermal layers, glands, and musculature underneath are fully shown to give a complete view of the skin's architecture. Salamanders have complex glands within the skin that are not found in mammals. They produce a surface slime that has toxins or low pH mucus that protects and aids in respiration of the skin. In figure H as well, we can even see the secretion of the gland onto the surface.

Discussion:

Through the past year and a half, I have become proficient enough in the histological method of observing tissue samples. I have learned the process of embedding the tissues within plastic that will be valuable to section with a microtone. I learned through a tremendous amount of practice how to work the microtome to get clean sections of my material. When sectioning, it's important to line up the glass cutter just right to get clean cuts and not damage and sample as well as the blade. With that, I have also completed the step of correcting my staining technique and have collected quality samples that will allow me to start creating my own slides on *Eurycea lucifuga*. When compared to the sparse literature on salamander skin, the technique for histological staining of my samples is consistent with that of previously studied lungless salamanders. From my work within the last year, I can now carry on my skills to start the qualitative portion of my research and continue this project within honors college and with the hope of publishing our findings in a peer-reviewed journal.

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REFERENCES

Cave salamander. Missouri Department of Conservation. (n.d.-b). https://mdc.mo.gov/discover-nature/field-guide/cave-salamander

Hamlett, Butch E.; Strecker, Andy G.; and Trauth, Stanley E. (1998) "Caudal Courtship Glands in the Cave Salamander, Eurycea lucifuga (Caudata: Plethodontidae)," Journal of the Arkansas Academy of Science: Vol. 52 , Article 21.-