# Preserving Microscopic Samples of Bearded Dragon (*Pogona Vitticeps*) Duodenum and Examining its Histology

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## Abstract

Becoming proficient in microscopy and understanding basic histology is a key part of becoming an effective researcher and biologist. Histology provides students with a visual image of many biological concepts, such as cell organelle composition and how cells function within the tissues of vertebrates. Reptilian histology is a particularly important area of microscopic study because so little is known about it. Providing histological data on reptiles benefits the academic community and our understanding of the physiology within reptiles. This project allowed me to become skilled in creating microscope slides and expanding my knowledge of the histological organization of the Bearded Dragon's digestive tract. Much of my research experience involved mastering the various techniques that are involved in histology research. I began by learning the techniques of fixation and embedding. I preserved specimens by saturating them in osmium tetroxide and embedding them in plastic resin. Next, I learned microtomy, using glass blades to slice thin sections of reptilian intestines. Finally, I practiced staining slides of intestines with dye so the fine details of the cells and tissues that make up the intestines could be visualized. The climax of my research experience was to compare my histological data to that of the existing literature. I found significantly smaller blood vessels in the villi than was previously reported. The surface of the villi was also more irregular than that observed by other researchers. Our staining procedure and the plastic sections provided much better differentiation between the columnar epithelial cells, brush border, and goblet cells. Plastic sections, instead of the previously used paraffin, allowed us to visualize more details subcellularly and will permit these preliminary findings to be expanded using transmission electron microscopy.

## Introduction

*Pogona Vitticeps,* also called the central bearded dragon, is a dusty tan lizard with spikes on its head. Its habitat is mainly in dry areas such as deserts and scrublands. It belongs to the family Agamidae and the order Squamata. It is omnivorous and is known to feed on vegetation, invertebrates such as insects, and vertebrates such as mice and other lizards.

The histology of this species' GI tract, particularly its small intestine, has been largely unstudied by the scientific community. Little is known about the composition of this tissue. A few researchers have taken micrographs of *Pogona Vitticeps* small intestine (Engelke et al 2020), but these specimens lack fine detail and high color contrast. The micrographs taken here will give us a better understanding of *Pogona Vitticeps* duodenum histology.

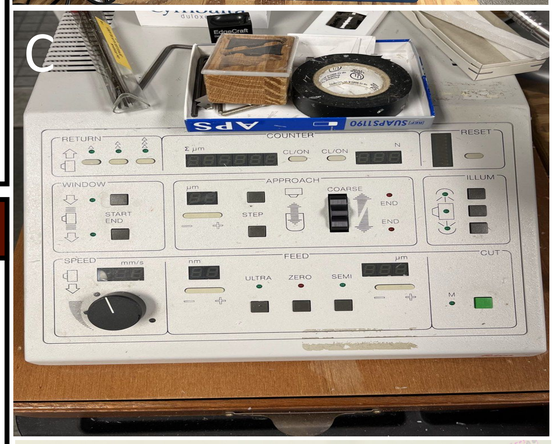
Obtaining a useful sample of duodenum required me to learn the complex steps of histology. Learning to do this process took over a year of work on my part as I could only dedicate several hours a week to this project, while dedicating my other time to my course work. The process of histology that involves embedding tissues in epoxy resin far exceeds the resolution at the tissue level within the digestive tract than earlier studies that relied on paraffin wax as their embedding medium.

## Materials and Methods

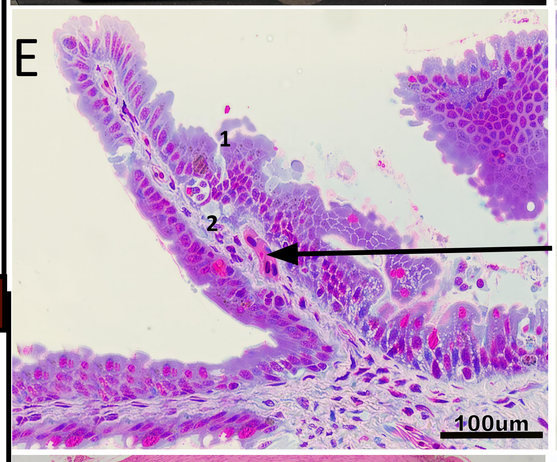
**Tissue Preparation for Electron Microscopy:** Duodenum samples were washed in cacodylate buffer 3 times for 10 minutes each. Samples were post-fixed in 2% osmium tetraoxide for 2 hours and then rinsed in DI water 3 times 15 minutes each. They were incubated in 1% uranyl acetate for 30 minutes and again rinsed in DI water 3 times 15 minutes each. Samples then were dehydrated in an ascending graded ethanol series. Plastic was made from 10 g ERL 4221, 8 g DER 736, and 25 g NSA. Duodenum samples were infiltrated first with a 1:1 solution of propylene oxide:plastic, then with a 1:2 solution of propylene oxide:plastic, and finally were placed in 100% plastic overnight. Samples were then incubated in new plastic resin for 2 hours and embedded in beam capsules before being cured in a Fisher isotemperature vacuum oven at 60° for 48 hours.

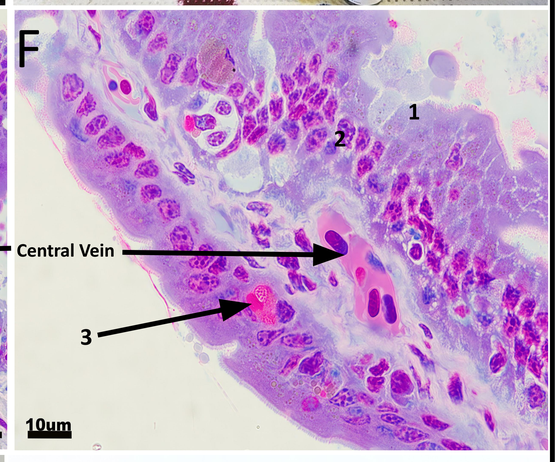
**Histological Analysis**: The embedded tissues were sectioned using a glass knife and then stained with toluidine blue and basic fuchsin. The process of sectioning was the longest and most arduous part of my research. This skill takes much time and patience to learn. Once, mastery of sectioning was determined by my advisor I then could move on to sectioning the digestive tract sections seen on this poster. The sections created by the glass knives were 2 to 3 um sections. We then stained them with a combination of Toluidine blue and Basic Fuchsin. Once I started producing good sections, then semithin sections at 05um were created using a Diatome diamond knife on a Reichert Ultracut S ultramicrotome. Images were taken on an Olympus compound microscope with a digital camera and manipulated and labelled with Adobe photoshop CS.

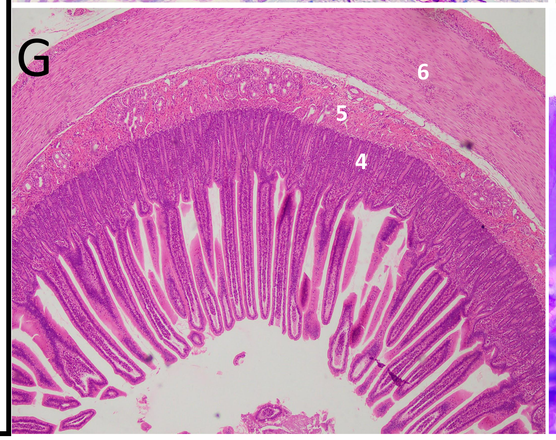


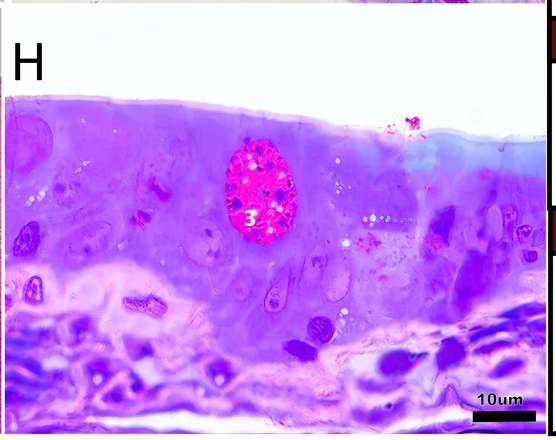







## Results and Discussion

**Figures A & C: Sectioning equipment**
**A.** The Reichert Ultracut S ultramicrotome was used to cut samples. Diamond/Glass knives were fitted to the machine and used to section pieces of the epoxy embedded samples. I practiced extensively on this machine for many months. I discovered how to use the settings so the samples were parallel to the glass blade. **C.** I familiarized myself with the forward and reverse step settings so I would not damage the sample or the knife. Finally, I made sure to cut into the plastic with the sharp portion of the knife. This resulted in highly detailed samples of small intestine.

**Figure B: Microscope**
This is an image of the Olympus light microscope used to take images. Micrographs for this presentation were taken at 40x and 100x magnification. Taking these micrographs required me to successfully operate the microscope and maintain its connection to a computer and work on making sure the parts of the tissue I wanted to present were in good focus.

**Figure D: Plastic materials**
Here is seen the compounds used to make plastic for embedding the duodenum samples. The measurements of these compounds were done carefully and precisely, as deviation from the instructions could result in our plastic being rendered useless.

**Figures E, F & H: Duodenum Villus**
These images show a duodenum villus at 40x magnification **(E)** and 100x magnification **(F)**. The third image **(H)** shows a 100x image of a goblet cell within a villus. Simple columnar epithelial cells line the villus. Beneath them lies the lamina propria. In the center is the central vein. The differentiation between the simple columnar epithelial cells (1), the lamina propria (2), and the central vein is clearly visible. The lamina propria is shown in greater detail than any previous micrography IN **F**. Mast cells (3) are often seen just under the epithelium. These cells are thought to be part of the inflammatory and immune response of the intestine. They are strategically placed near the epithelium and blood vessels so that they can release histamine when stimulated.

**Figure G: Small intestine**
This image is a 20x power micrograph of the *Pogona Vitticeps'* small intestine. The villi are visible as finger-like projections in the lower middle of the micrograph. Surrounding the villi is the muscularis mucosa (4), the submucosa (5), and the smooth muscle of the small intestine (6).

**Discussion**
The histology of *Pogona Vitticeps* has not been extensively studied before, so these micrographs give us a more complete picture of its anatomy and physiology. We were able to obtain more detailed images than previous studied, which will give further insights into the process of digestion and a model to compare to other lizard GI tracts. Some variations from current research should be noted: smaller central veins were observed, as was the presence of mast cells in the duodenum. This project allowed me to become familiar with biological preservation/histology techniques for microscope slides. I was able to explore the processes of embedding, sectioning, and staining, and become comfortable performing these procedures on my own. I now have a greater grasp of both reptile histology and the process, which increases my understanding of how the architecture of a tissue can help ID that tissues functions.

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## References

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