

Analysis of the Function of Small  
Intestinal Aggregates of the  
*Necturus* Based on Old Classical  
and Recent Literature Data

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# Objectives

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- To analyze classical literature surrounding the possible functions of the cellular aggregates found in the small intestine of the *Necturus*
- To understand how the limitations of the scientific technology of the period may have led to inconclusive or incorrect conclusions
- To understand modern scientific techniques that could now be used to assist in answering this question
- To use the information gained from these modern techniques regarding related species to to perform a comparative analysis to reach a possible conclusion

# The Necturus

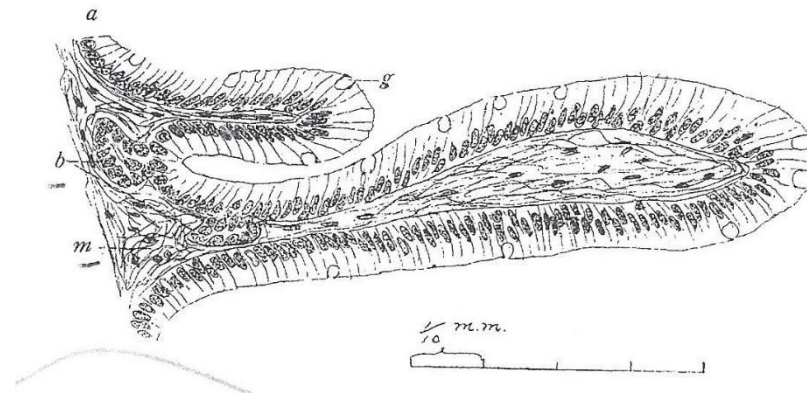
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- Also known as the mudpuppy, is an amphibious animal related to frogs
- Historically an important model organisms for labs, but is now protected due to population decline
  - Were vertebrate organisms that were cheap to maintain and had few regulations in place regarding their treatment
  - Has led to a great body of work surrounding them that can no longer be explored directly
- One classic argument that was never resolved was whether the cellular aggregates seen in histological preparations of their intestines are glandular or proliferative in function

# Cellular Aggregates

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- The structures in question appear to extend from the mucosa of the intestine
- Tend to sit along the invaginated folds of the intestine
- If glandular- They should have a luminal surface and space to hold a product, as well as a duct to the intestinal lumen through which to secrete their product
- If proliferative- They should have a higher rate of mitosis than the surrounding tissue (measured by mitotic index), and there should be movement of new cells out of aggregate and into mucosa
  - Cells populating the aggregate should be stem cells
- In the drawing, the aggregates are labelled “b”



(Figure taken from Kingsbury, 1894).

# Stem Cells

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- Undifferentiated cells that can mature into multiple types of mature cells based on the signaling pathways they interact with
- Main classifications
  - Multipotent- Able to differentiate into several different types of mature cells
  - Pluripotent- Able to differentiate into any of the cell types that make up the body
  - Totipotent- Able to differentiate into any of the cell types that make up the body, plus extraembryonic or placental, cells
- Function to develop tissue types in growing organisms and maintain cell populations in developed tissues (process known as proliferation)
  - Stem cells can divide and differentiate only a portion of the daughter cells, thus maintaining the reserve of stem cells and creating new cells to replace the cells in the tissue that have died

# Problems Identifying SC

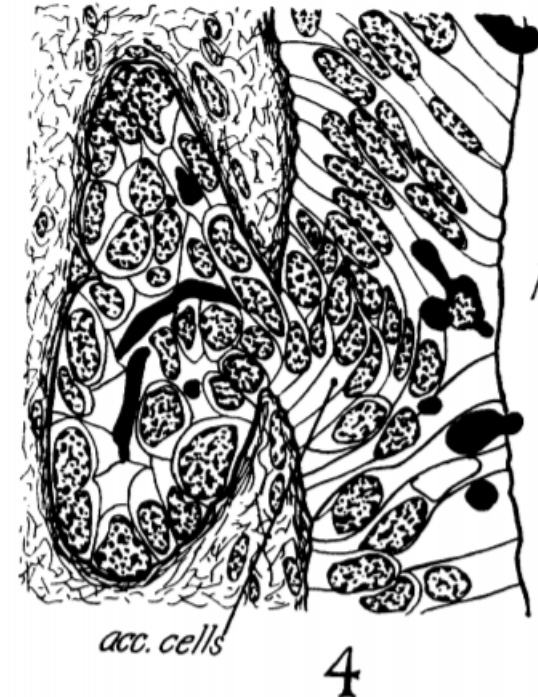
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- Individual cells are morphologically similar to many other cell types
- Groups of different types of stem cells don't exhibit a rigid standard morphology
- Many differentiated cells are identified by a particular function of biological pathway they participate in
  - Because their function is to become these other cells, stem cells don't yet display these easily identifiable markers

# Classical Literature

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- Argue glandular function
  - Hoffman (1878)- Claimed his preparations showed glandular lumen
  - Kingsbury (1894)- Claimed his preparations showed glandular lumen
  - Bates (1904)- Argued other similar species had been shown to have intestinal glands
- Argue proliferative function
  - Mead (1916), Patton (1960), and Nicholas (1894)
  - Collectively argue that their preparations lack evidence of lumen or ducts
  - Also note that the aggregate has a much higher mitotic index than the rest of the intestinal mucosa
- Argue dual function
  - Sarcedotti (1894), Dawson (1927), Goldsmith (1929), and Bizzozero (1982)
  - Argue that the aggregates switch back and forth between the two functions based on the needs of the intestine
  - Seek to appease the fact that both sides seem to be making valid and reputable arguments

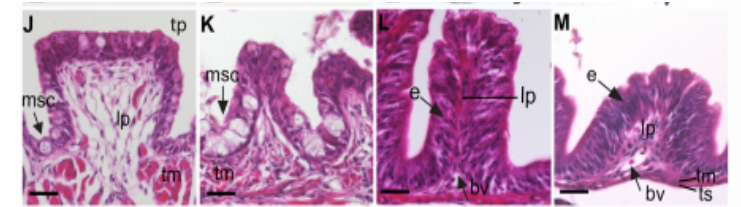


(Figure taken from Dawson, 1927).

# Classic Techniques

## ■ H&E (Hematoxylin & eosin)-

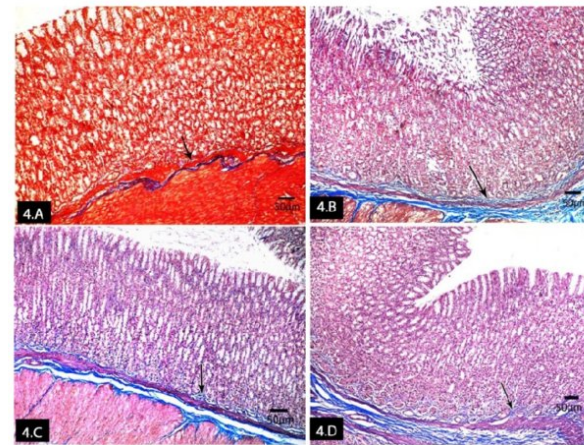
- Hematoxylin- Basic stain with a positive charge that stains nucleic acids a dark purple/blue due to its interaction with the negative phosphate backbone
- Eosin- Acidic stain with a negative charge that stains the cytoplasmic proteins pink since cytoplasmic proteins tend to be basic
- High degree of detail and clarity, allows for calculation of mitotic index



(Figure taken from Aghaallaei et al., 2016).

## ■ Mallory's trichrome complex-

- Aniline blue- Stains collagen a deep blue color
- Acid fuchsin- Stains cytoplasm and nuclei red
- Orange G.- Stains red blood cells orange



(Figure taken from Ahmed et al., 2015).



# Limitations

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- Could not detect signaling pathways or genetic expression
- Resolution much lower than techniques that exist today
- Before modern imaging preparations had to be hand drawn to show others
- Improvements in instruments, chemicals, and procedures create preparations with fewer artefacts
  - Artefact- An artificial structure or tissue alteration seen on a histological preparation resulting from the preparation process

# New Techniques

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- Karnovsky's fixative
  - Developed 1965 to fix tissue for use in electron microscopy
  - Preserves the morphological and chemical integrity of the tissue using both formaldehyde and glutaraldehyde as embalming chemicals
  - Formaldehyde is smaller and is able to permeate the tissue faster and create a weaker hold to keep the tissue in place
  - The larger glutaraldehyde molecule diffuse more slowly, but create a stronger fixation
  - Work by crosslinking polymers to each other via ionic and covalent bonding
  - Preserves microtubules especially well, which is critical for determining a mitotic index
- Embedding in Durcupan
  - Unlike its predecessor, paraffin, it doesn't require an organic solvent that can negatively affect the integrity of the tissue

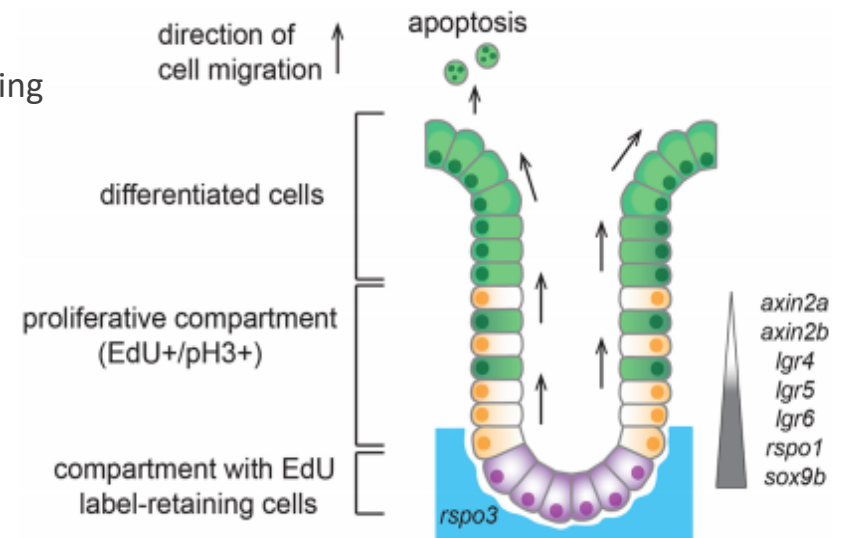
# New Techniques

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- BrDU (5-Bromo-2-deoxyuridine)
  - BrDU acts as a thymidine analog, that when injected, will be incorporated into the DNA of cells undergoing DNA synthesis for division
  - The BrDU can then be visualized with antibodies and fluorescent microscopy
  - Will show not only which cells were dividing, but also the lineage of dividing cells if left in the tissue for multiple generations of cell division
- Signaling markers
  - Discovered by studying the crypts of Lieberkühn in mice, which are intestinal aggregates with confirmed stem cell function
  - Four main signaling cascades have been identified as different between stem cells and mature epithelial cells- Wnt, Notch, Indian Hedgehog (Ihh), and BMP
  - Are highly conserved pathways, that if shown to operate in *Necturus* aggregates, would be evidence in favor of their function being proliferative

# New Techniques

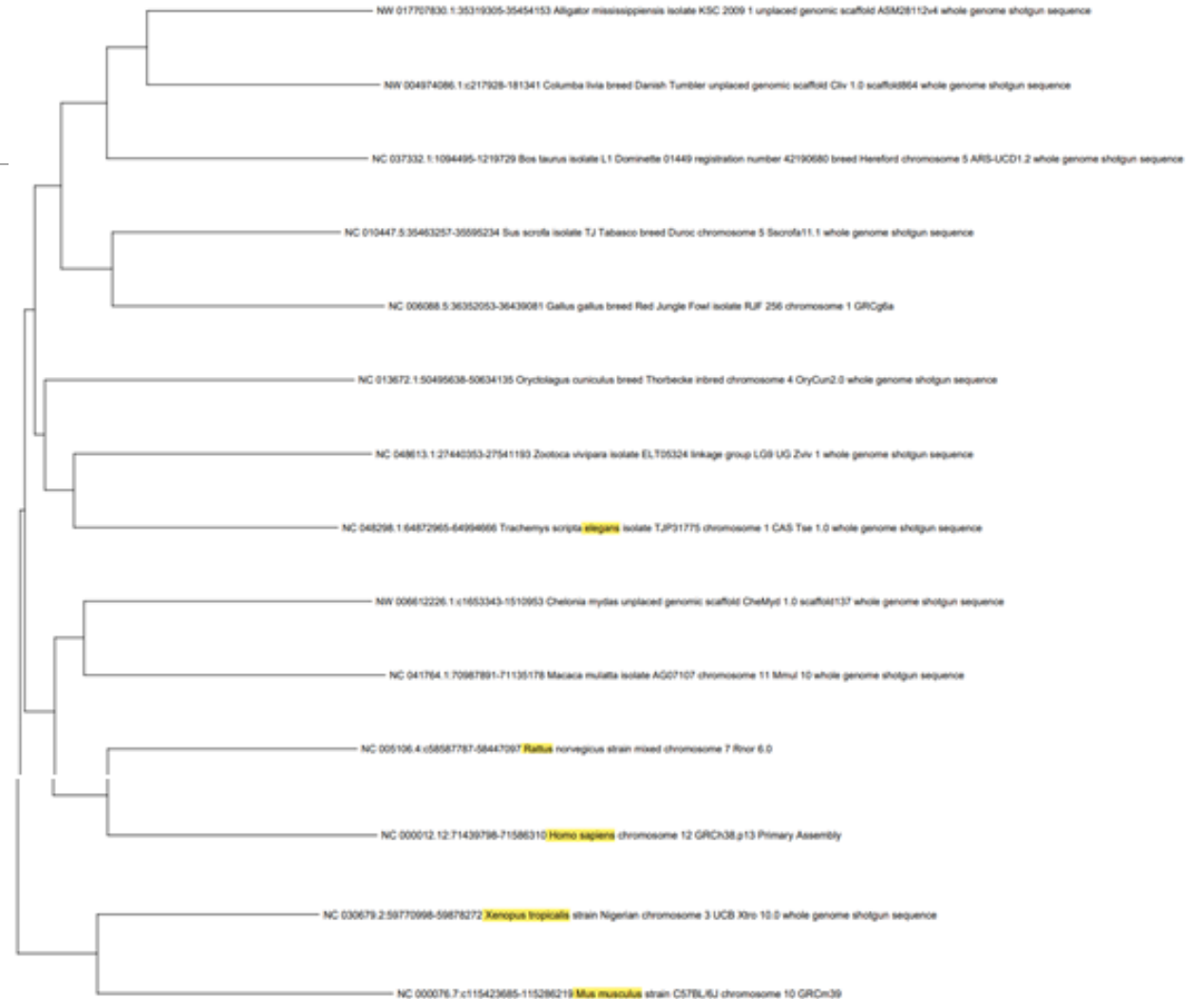
- Gene expression markers
  - Similar to signaling cascades, there have been several genes whose activity has been linked with stem cell function
  - *Lgr5*, *Bmi*, *DCAMKL-1*, and *Sox9*
    - Identified to be functional in stem cells, but have little to no function in the surrounding epithelial tissue
  - The schematic shows high levels of these markers at the base of the crypt
  - The level of expression decreases as you move up the crypt into the mucosa as the stem cells there tend to be more differentiated



(Figure taken from Aghaallaei et al., 2016)

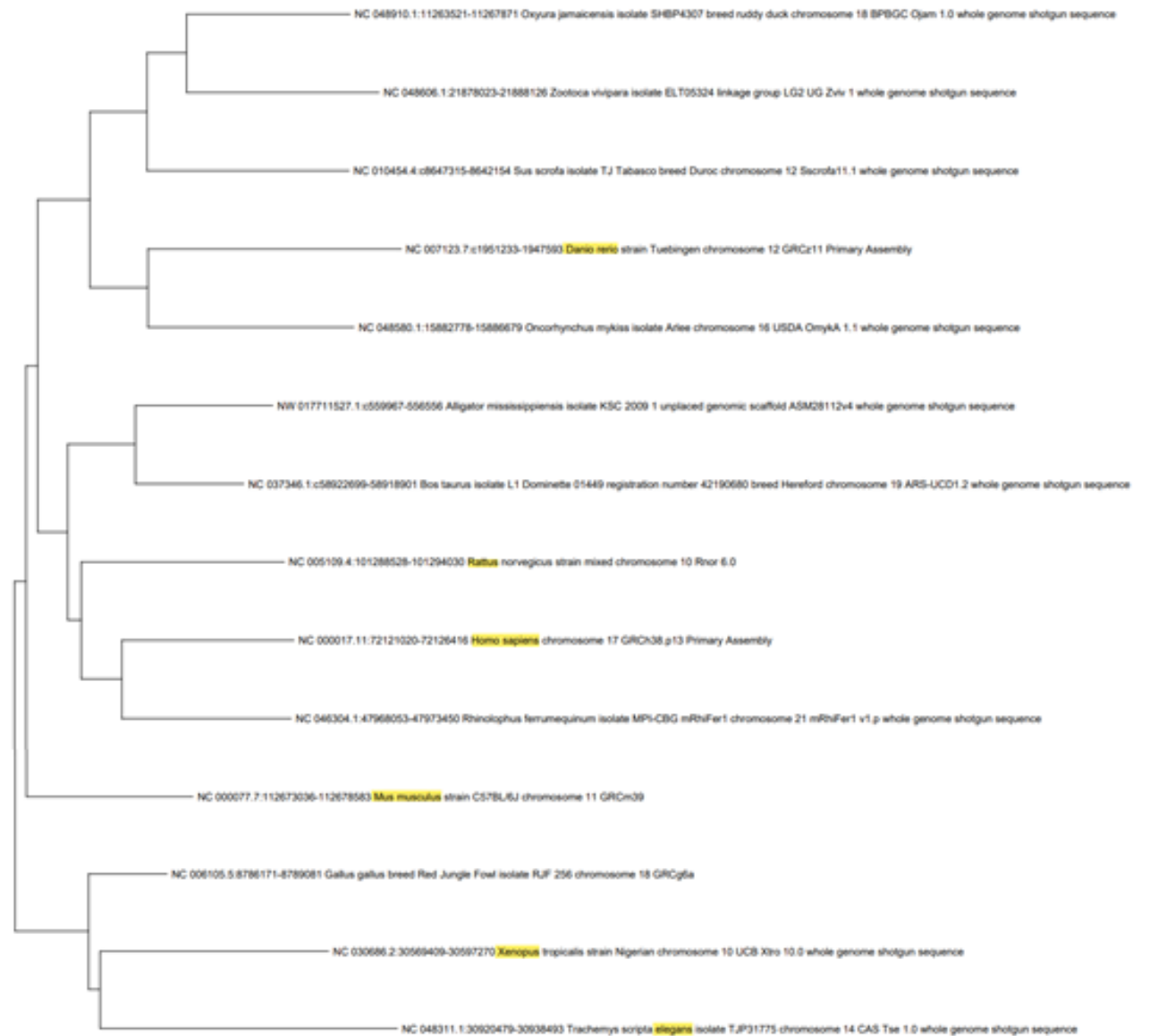
# New Techniques

- Phylogenetic comparison- Using gene sequencing to compare how similar a given gene is across species
- Created using the NCBI database records of the *Lgr5* gene
  - *Necturus* is not fully sequenced, so its relative the frog was used in its place
- Highlighted species being worm, rat, human, frog, and mouse moving from top to bottom
- Shows the species with most sequence homology to the frog is the mouse
  - Mice are known to have proliferative intestinal crypts



# New Techniques

- Created using the NCBI database records of the *Sox9* gene
- Highlighted species being zebrafish, rat, human, mouse, frog, and worm moving from top to bottom.
- Important to note- These trees don't represent conclusive data, but show the possibility of a certain function via the species' relationships



# Conclusion

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- Recent literature has given us a detailed understanding of the intestinal makeup of other close species, which could be compared to historical preparations of the *Necturus*.
- In mammals, using visualization of signaling pathways and migration patterns, intestinal aggregates were shown to be crypts of Lieberkühn
- The aggregates of the *Necturus* are comparable in morphology, location, and pattern of occurrence to the crypts of Lieberkühn
- In mice, similar patterns of mitosis were found as noted in the *Necturus*- High mitotic index in the aggregates, and very low mitotic index and epithelium
- From this we conclude that the “aggregates” are most likely indeed equivalent to the Crypts of Lieberkühn in the mammalian small intestine

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