Regulation of Cell Volume in Alligator RBCs

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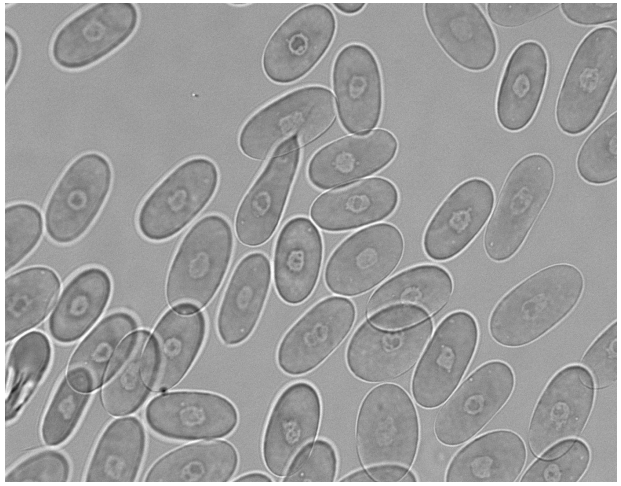
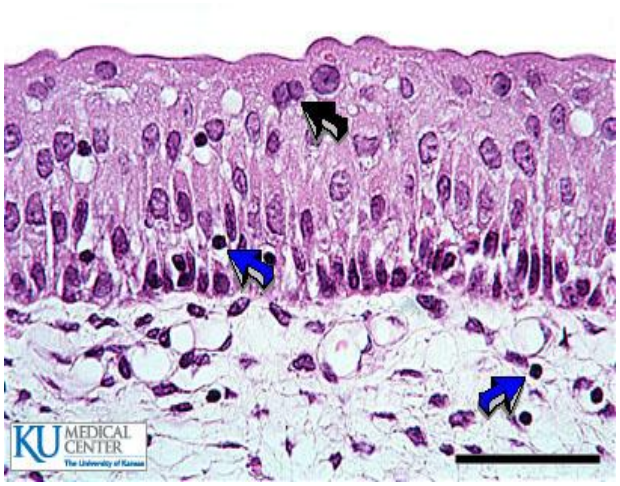
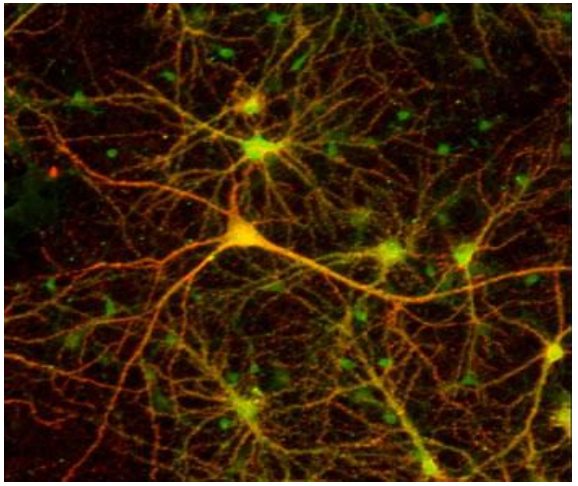
It's All About Cells

- Fundamental unit of life
- All organisms are made of one or more cells
- Humans contain 20-30 trillion red blood cells!





http://www.kumc.edu/instruction/medicine/anatomy/histoweb/urinary/renal19.htm



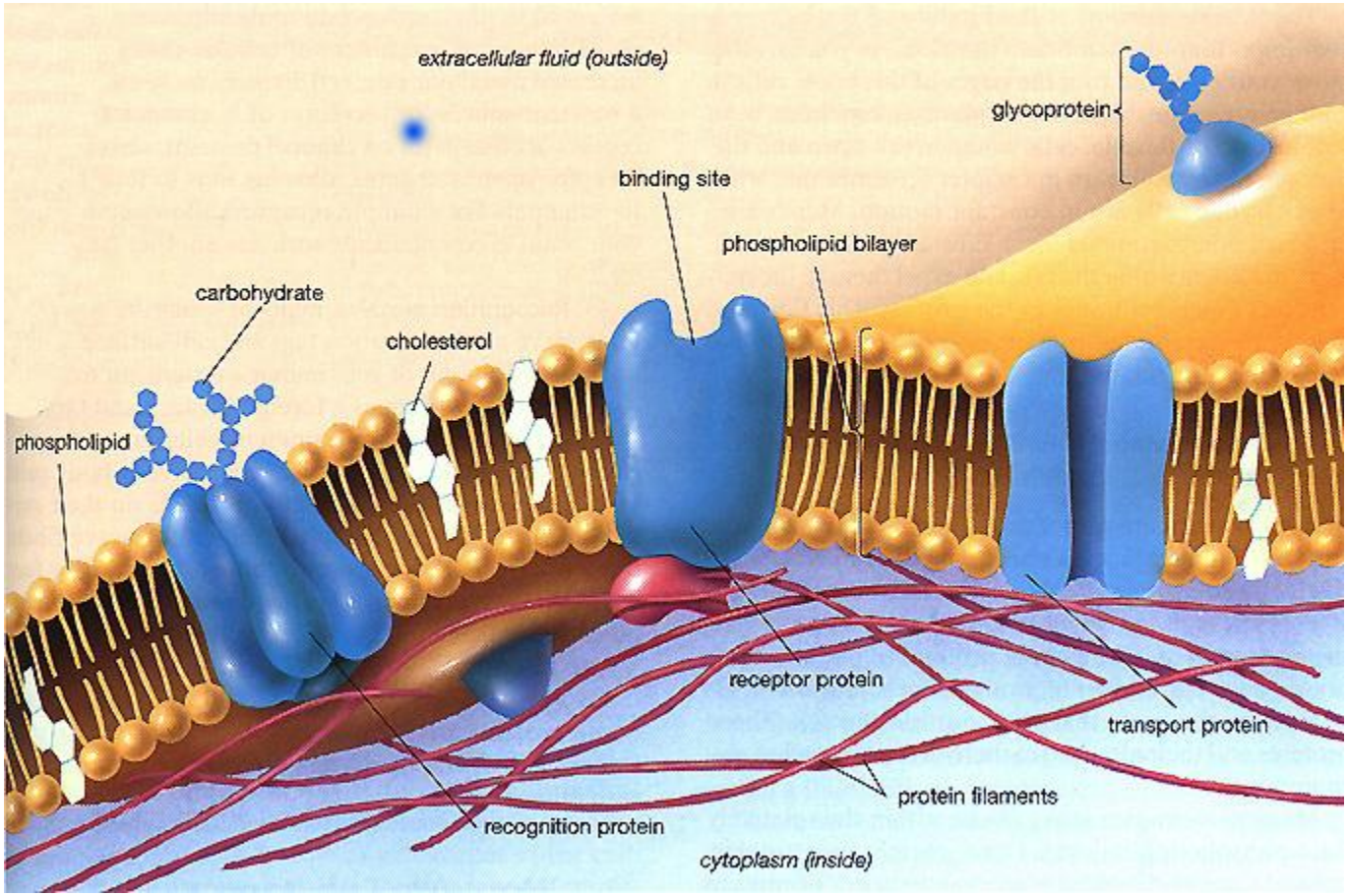
http://www.topnews.in/health/disruption-nerve-cells-may-lead-parkinsons-212005

Membrane-Bound or Bust

- Encloses all cells
- Regulates cell content
- Maintains steady-state conditions (homoeostasis)
- Must be selectively permeable

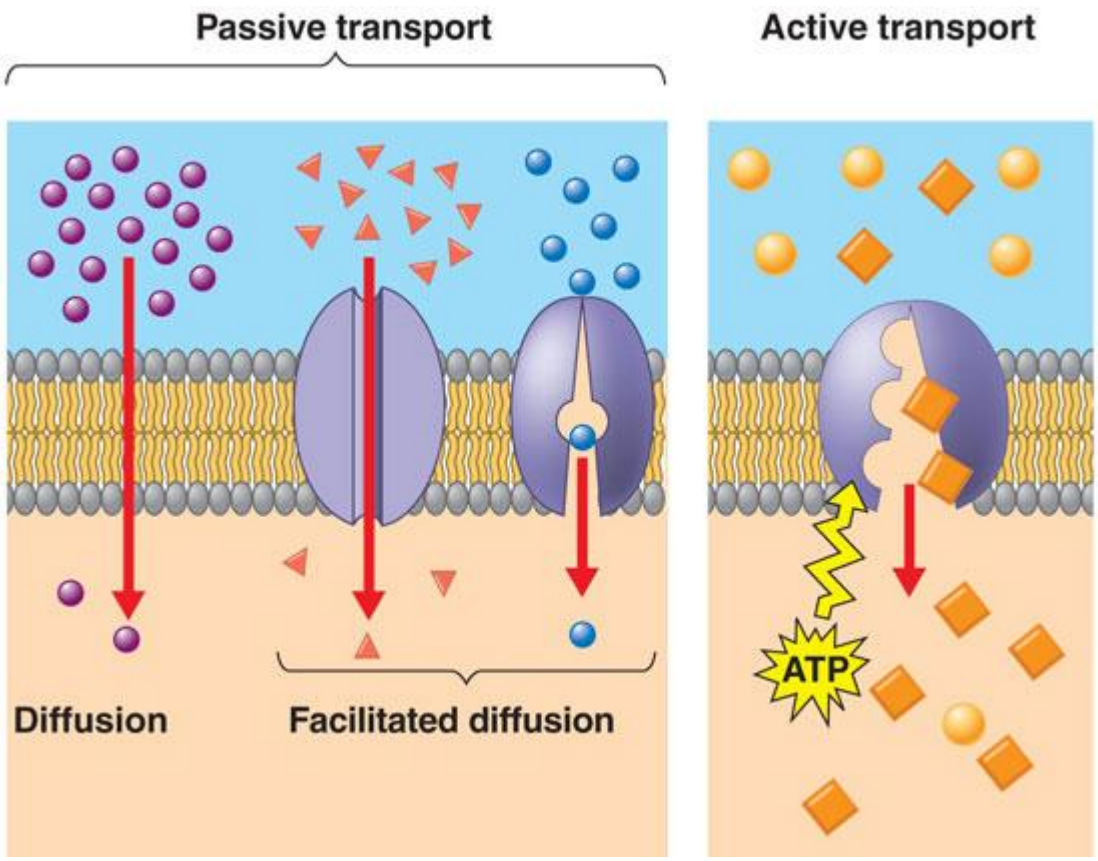
http://www.stemcellsinc.com/science/stem-cells-101.htm

The "Fluid Mosaic Model"



http://alevelnotes.com/The-Fluid-Mosaic-Model/129

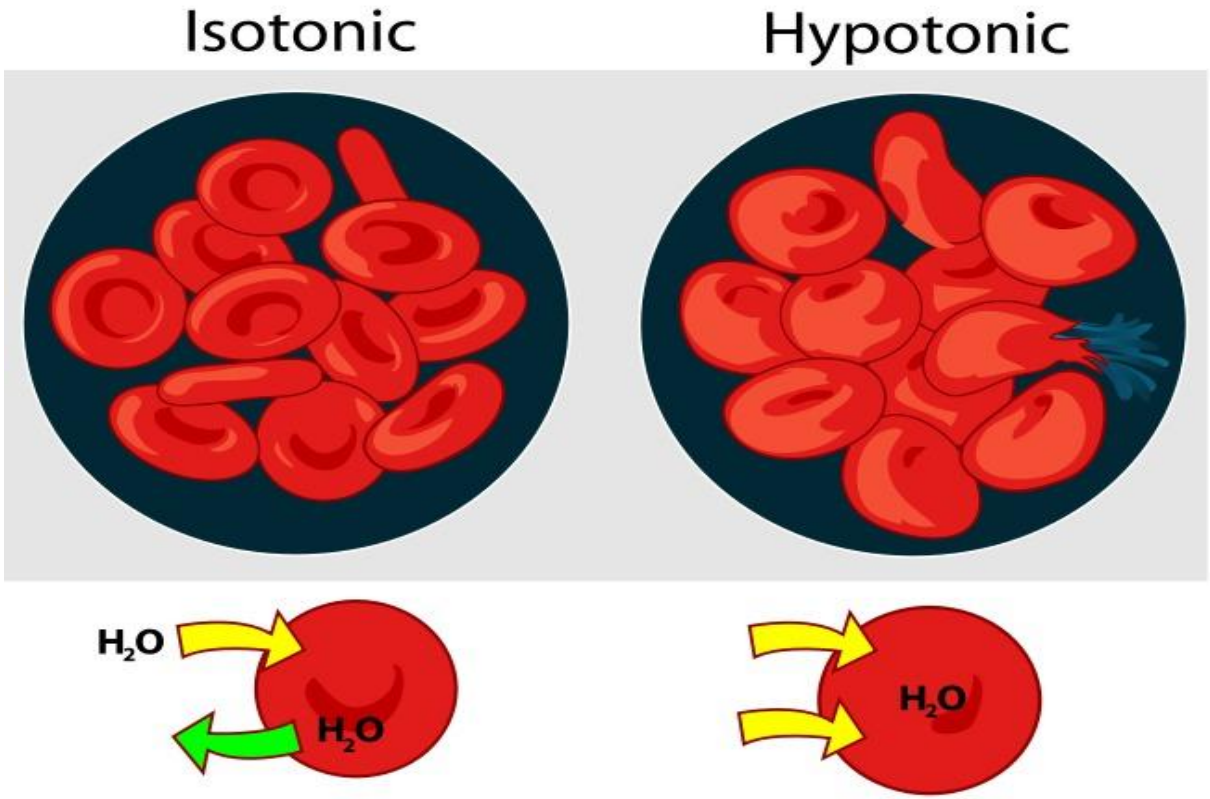
I Get By With A Little Help From... Ion Channels



http://biogeonerd.blogspot.com/2013/01/membrane-transport-and-osmotic-pressure.html

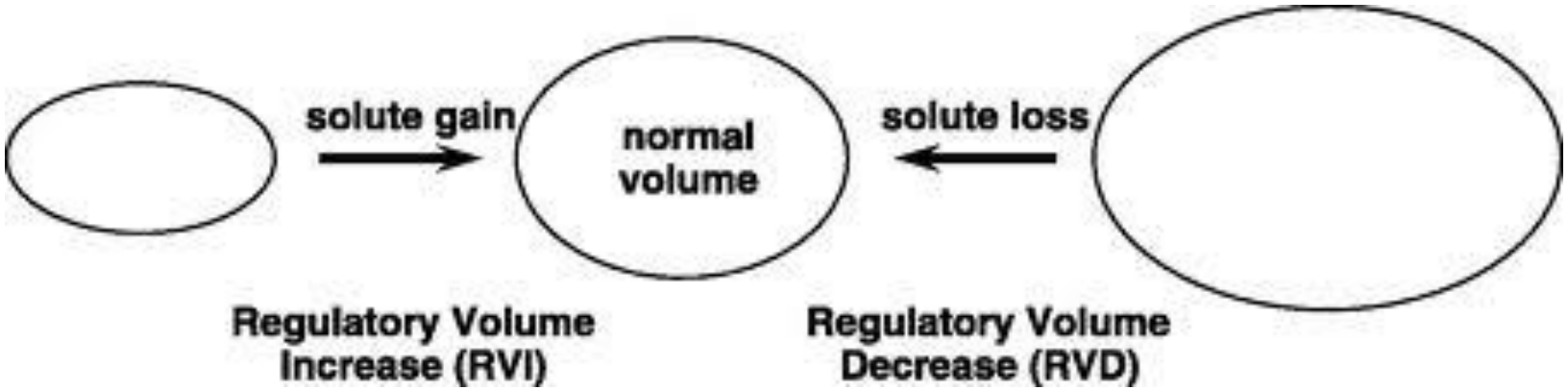
- Membranes act as barriers
- Ion channels regulate what particles enter or exit the cell (based on concentration)

Osmosis and Red Blood Cells



http://chemistry.about.com/od/imagesclipartstructures/ig/Science-Clipart/Osmosis---Blood-Cells.htm

Regulatory Volume Decrease

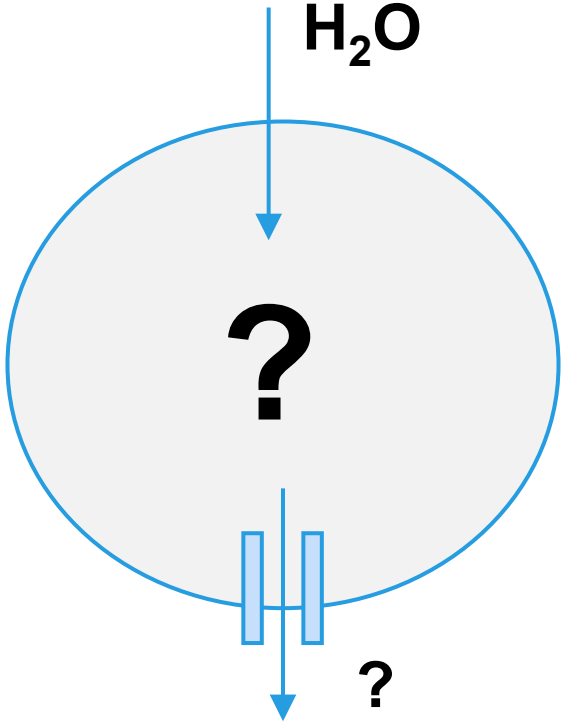


http://advan.physiology.org/content/28/4/155/F2.expansion

- **Acute swelling triggers mechanisms to push particles (solutes) OUT of the cell**
- Water follows by diffusion
- Cell volume decreases towards initial volume

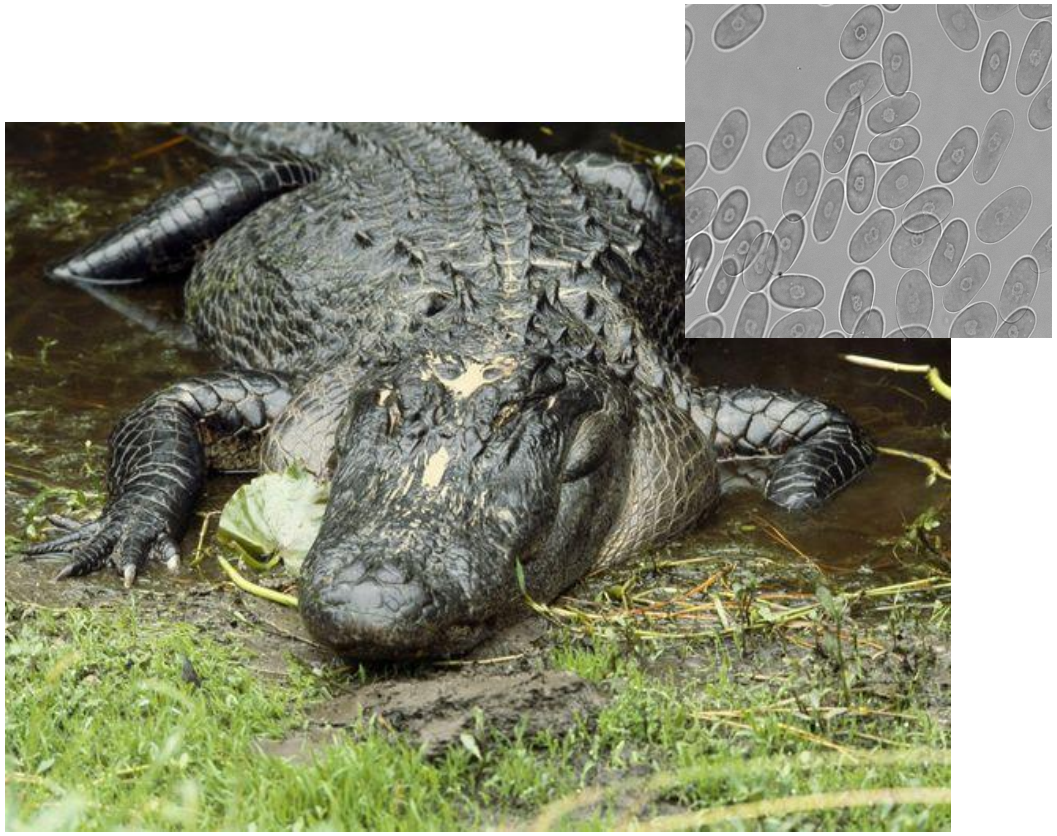
Gap In Knowledge

- What solutes are lost during RVD?
- By what mechanisms are the solutes lost?
- How are these mechanisms controlled?



Some Models Don't Need a Runway

Do American alligator RBCs possess a robust RVD response due to reduced kidney function during hibernation?



http://advan.physiology.org/content/28/4/155/F2.expansion

Method to Our Madness

Coulter Counter

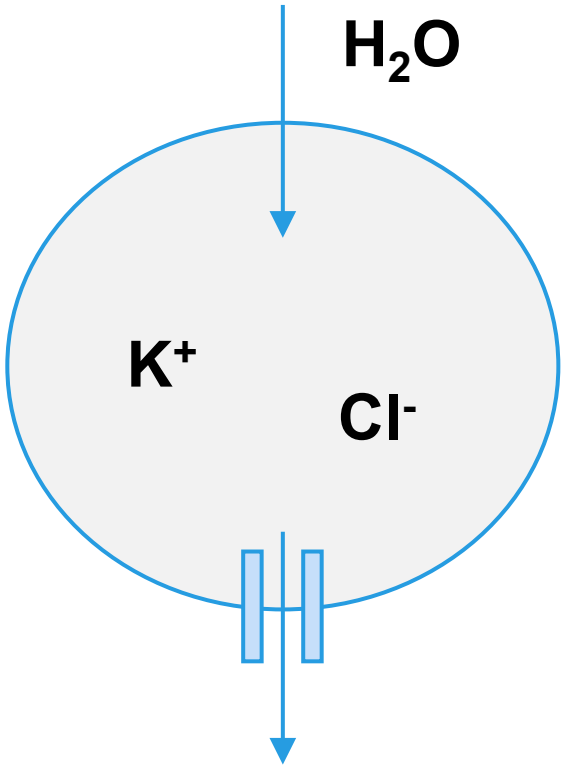
http://www.immunotox.com/Facilities/CoulterCounter.html

Ringer Solutions

https://www.doccheckshop.com/Practice/Injection-Infusion/Other-injection-and-infusion/BBraun-Ringer-Solution.html

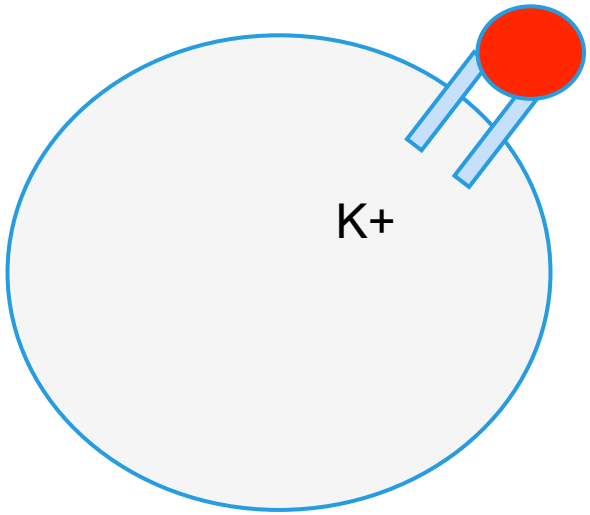
Losing My KCl

- Cells contain a large quantity of potassium and chloride
- Unlike most cells, chloride channels are typically open in RBCs

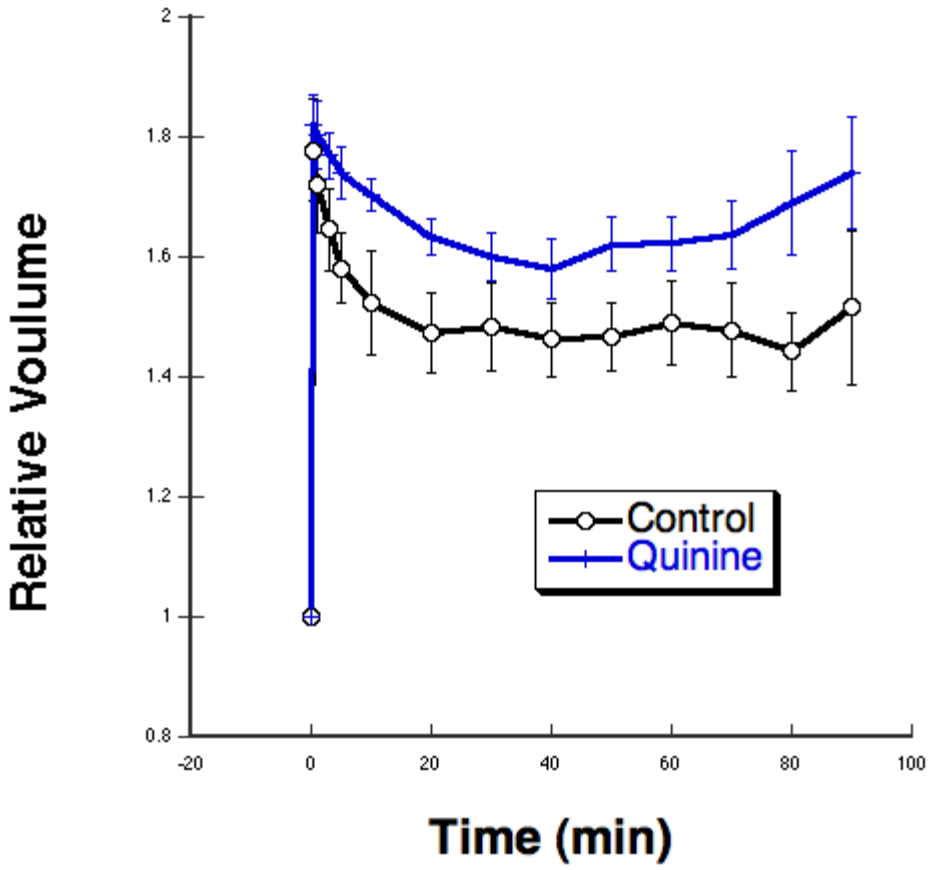


Hypothesis #1

- If potassium channels are necessary for RVD, then a drug (quinine) that blocks potassium channels should inhibit a RVD response.

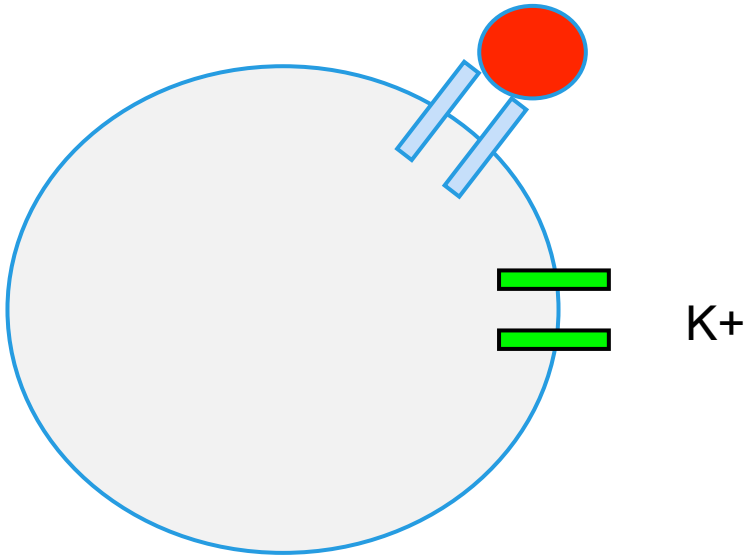


RVD Relies on Potassium Efflux

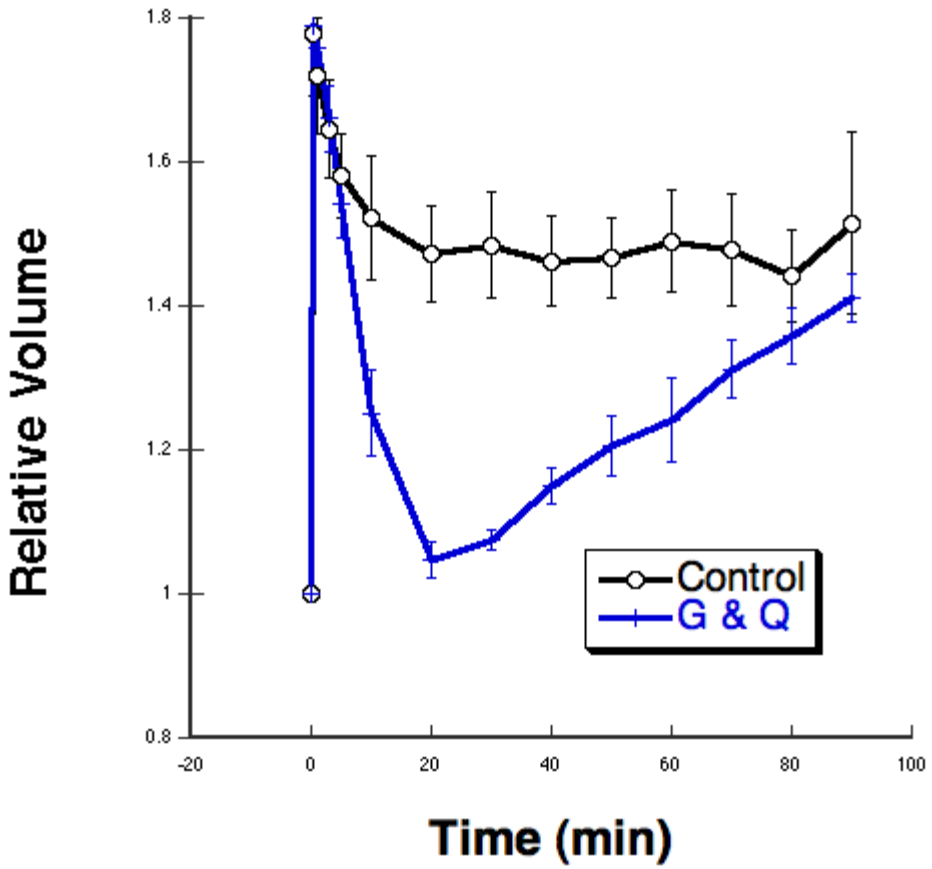


Hypothesis #2

- If potassium efflux was blocked by quinine, then gramicidin should prevent the inhibitory effect of quinine.

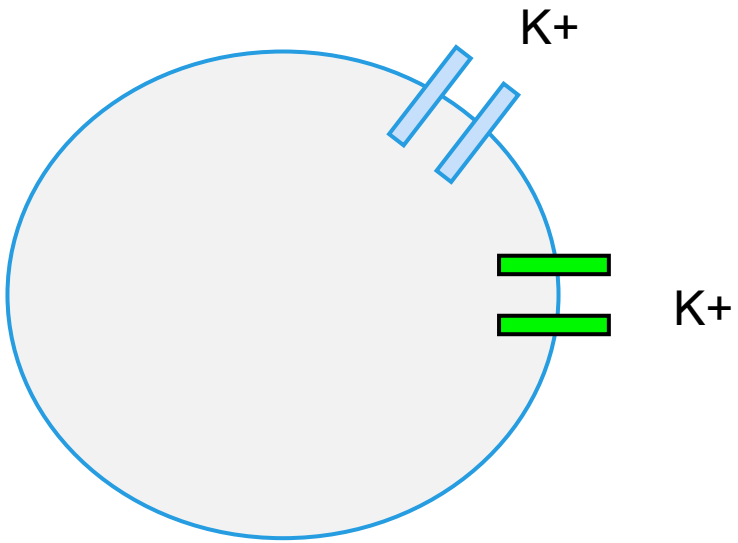


RVD Relies on Potassium Efflux

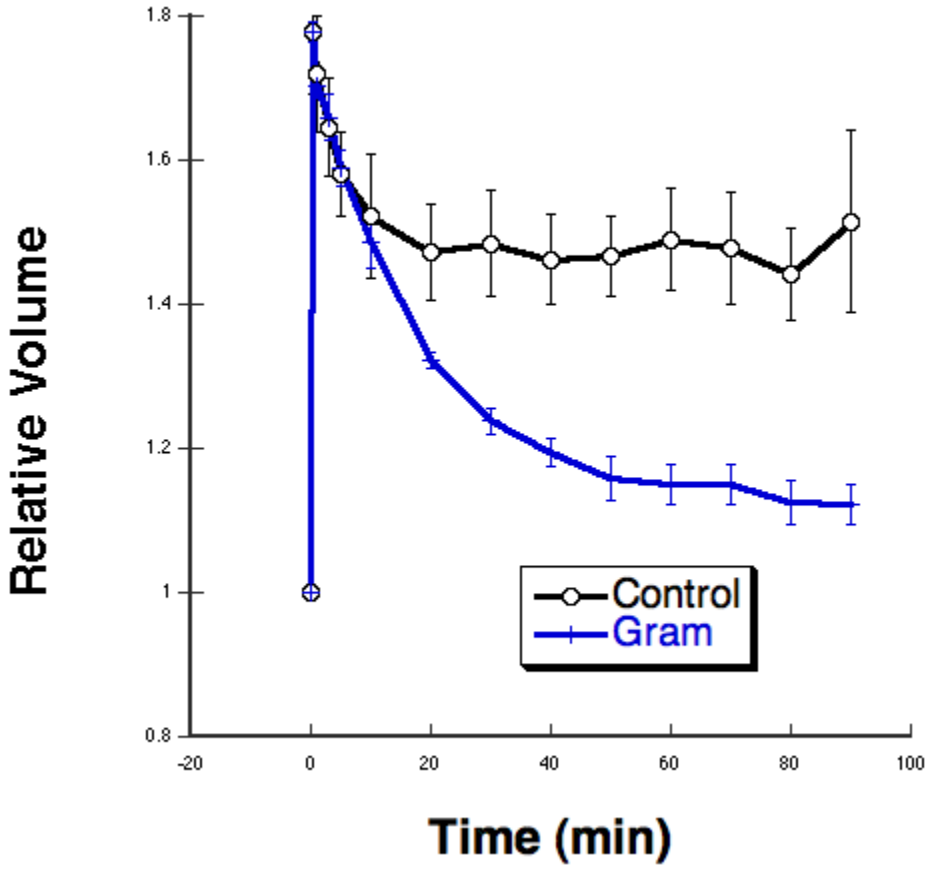


Hypothesis #3

- Additionally, if potassium efflux is a rate limiting step, then adding gramicidin (which creates more K+ channels) should produce a faster RVD response.

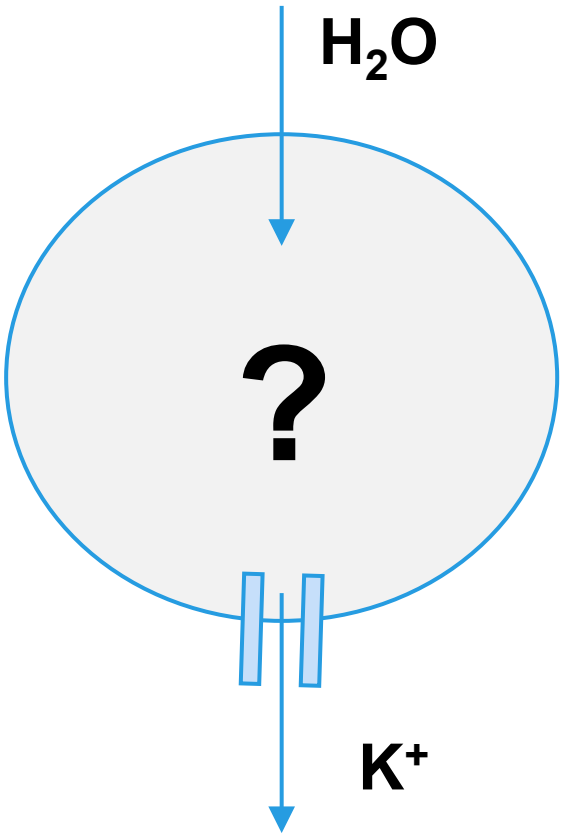


Potassium Efflux is Rate Limiting



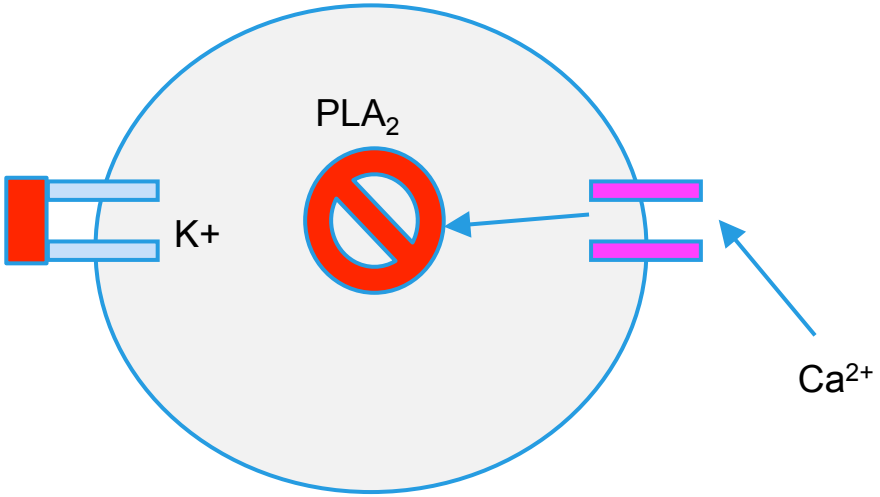
Gap In Knowledge II

There has to be a signal transduction process that opens potassium channels...

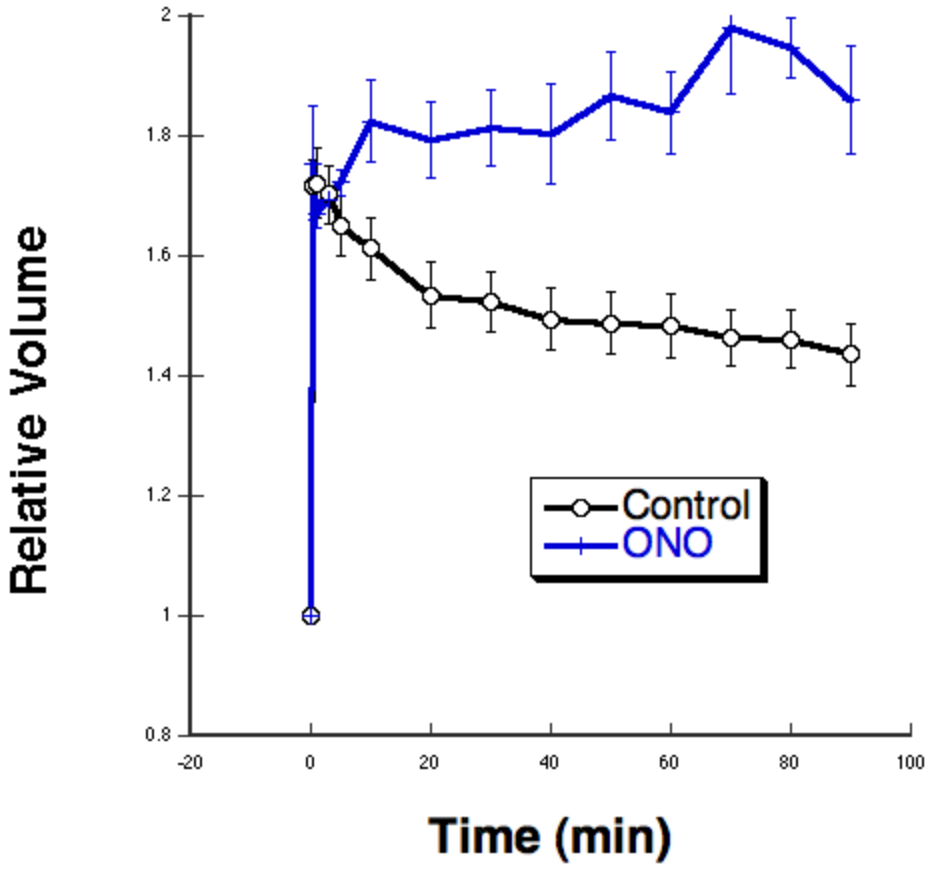


Hypothesis #4

- If PLA_2 is necessary for the cascading reaction leading to K^+ efflux, then ONO (which blocks PLA_2) should <u>inhibit</u> a RVD response.

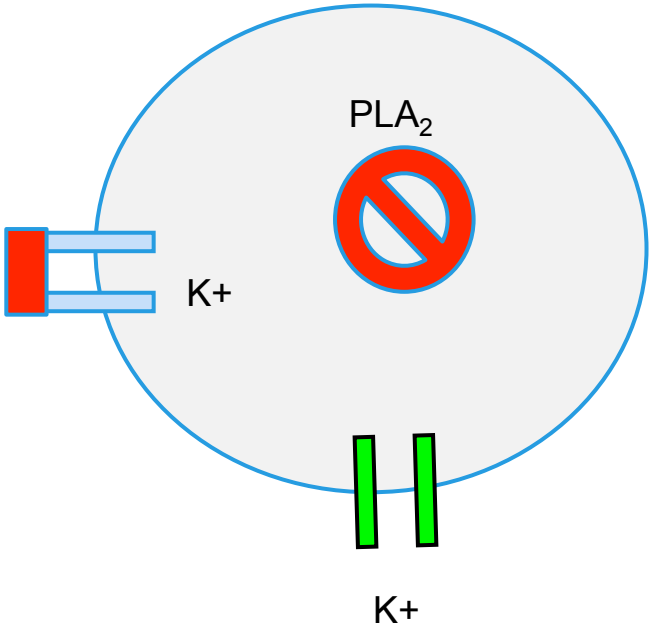


RVD Relies on PLA_2 Enzyme

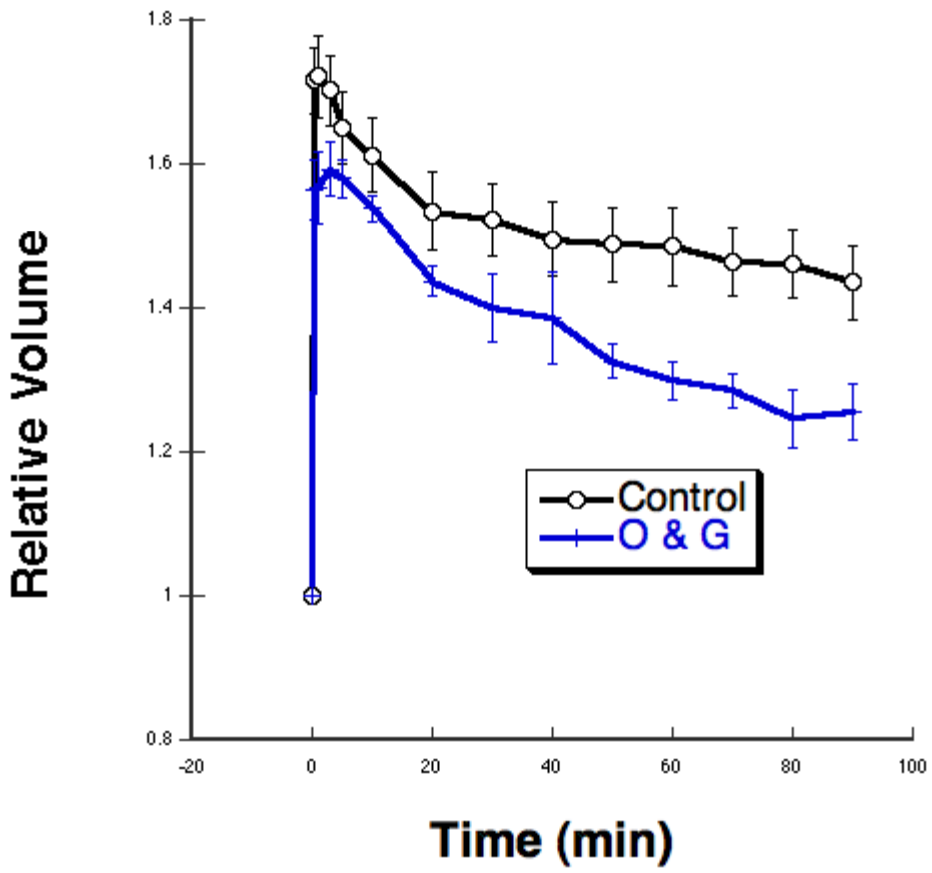


Hypothesis #5

- If gramicidin adds K+ channels to the membrane, then the addition of gramicidin should <u>prevent</u> the inhibitory effect of ONO (consistent with PLA_2 being necessary to open K^+ channels).



RVD Relies on PLA_2 Enzyme



The Take-Home Message

- Potassium efflux is necessary for RVD
- Potassium efflux is the rate limiting step
- Potassium efflux depends on the activation of the enzyme PLA_2

Back to the Future (Studies) I

- Determine what type of K+ channel is involved in RVD (e.g. patch clamp technique, pharmacological studies)
- Further examine the role of arachidonic acid and its metabolites in RVD
- Determine what caused the increased cell volume that occurred around 60 minutes

Back the Future (Studies) II

- Determine the role of calcium in RVD (e.g. chelate extracellular calcium with EGTA, fluorescence microscopy)
- Examine the potential role of amino acid efflux in RVD (e.g. taurine)
- Examine the potential role of chloride channels during RVD (patch clamp technique)

Acknowledgements

- **Professor Light:** Thank you for your guidance, patience, wit, and constant reminders to put safety first and/or to not put pens in our mouths.
- **Richter Committee & Professor Moroney:** Thank you for your time and providing us with this wonderful opportunity!
- **Fellow Richters:** Thank you for your moral support and the memorable times.

Got Questions?

- We hopefully have answers
- Or some good postulations