Measuring water absorption in the digestive tract of the little skate, *Leucoraja erinacea*

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Sharks, skates and rays appeared approximately 500 million years ago and have undergone very little morphological change since. Thus, studying these animals provides a snapshot into animal history. We are interested in finding the evolutionary origin of the colon, a digestive tract organ the main function of which is to absorb water and prevent dehydration. We found that the skate intestine has the ability to absorb water. This is an important clue to understanding the origin of the colon and the adaptation of vertebrate animals from aquatic to terrestrial life.

Marine elasmobranchs have high levels of urea and TMAO (trimethylamine oxide) in their body fluids to remain isotonic relative to their ocean environment. As a result, elasmobranchs living in seawater experience very little to no water loss due to osmosis [8]. Water absorbed through the gills is countered by an increase in renal excretion. Thus, marine elasmobranchs do not actively drink seawater to maintain osmotic consistency as is experienced by marine teleosts [2,3]. Despite this, we have histological and immunohistochemical evidence that a region of the spiral intestine in marine elasmobranchs can, in fact, absorb water. In the vertebrate digestive tract, acid mucins are associated with regions of high water absorption [4,6]. High levels of acid mucins are found in the distal region of the spiral intestine, suggesting that this region may absorb water [7]. In addition, water-selective aquaporins are expressed in the spiral intestine [5]. Together, these data suggest that despite the unique osmoregulatory nature of elasmobranchs, they may have regions of their digestive tract that can absorb water. To test this hypothesis, we investigated the ability of the little skate, *Leucoraja erinacea*, digestive tract to absorb water by directly measuring water absorption in different regions of the digestive tract under different osmotic pressures.

To measure water absorption, digestive tracts from adult male little skates were harvested and flushed clean with running marine water followed by Elasmobranch Ringer's solution. The stomach and spiral intestine were dissected from the digestive tract, and the intestine was further divided into proximal and distal halves. Each tissue section was filled with Elasmobranch Ringer's solution to a pressure of 1.0 kPa, tied at each end, and measured for length (cm) and weight (mg). The tissue was kept in a stirring bath of Elasmobranch Ringer's with an aerator. The intestine or stomach was weighed every 30 minutes for a total of 3 hours. The loss of mass corresponded to the amount of water absorbed from the lumen over a given time. For experiments examining the effects of osmotic pressure on water absorption, the pressure of Ringer's inside the tissue was either maintained at 1.0 kPa or increased to 3.0 kPa. This protocol was adapted from a similar method reported on *Anguilla japonica* [1].

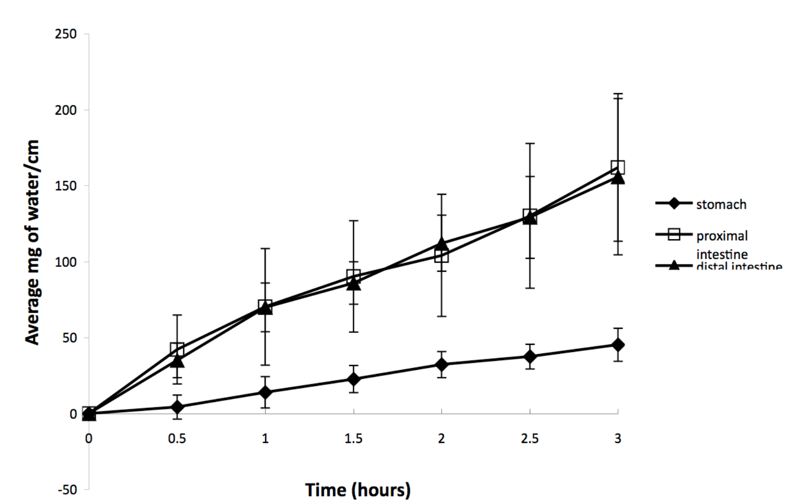

Figure 1. Water absorption in the stomach, proximal and distal spiral intestines of *L. erinacea* at 1.0 kPa. The volume of water absorbed from the lumen was calculated as the loss of mass of the intestine. Net water absorption was determined for each time internal from time zero. Graphed is the net water absorbed per length of tissue (cm) over 3 hours.

Water absorption occurs in both the stomach and spiral intestine of *L. erinacea*. Net water absorption is linear with time and is higher in the spiral intestine than the stomach (Fig. 1). There is no significant difference in water absorption between the proximal and distal regions of the spiral intestine (Fig. 1). To determine if water absorption in the spiral intestine is pressure-dependent, we investigated the amount of water absorbed in spiral intestines at 1.0 kPa and 3.0 kPa. Interestingly, water absorption is unaffected by an increase in pressure inside the intestine (Fig. 2). This suggests that water transport across the membrane is not due to changes in osmotic pressure, but is the result of facilitated diffusion.

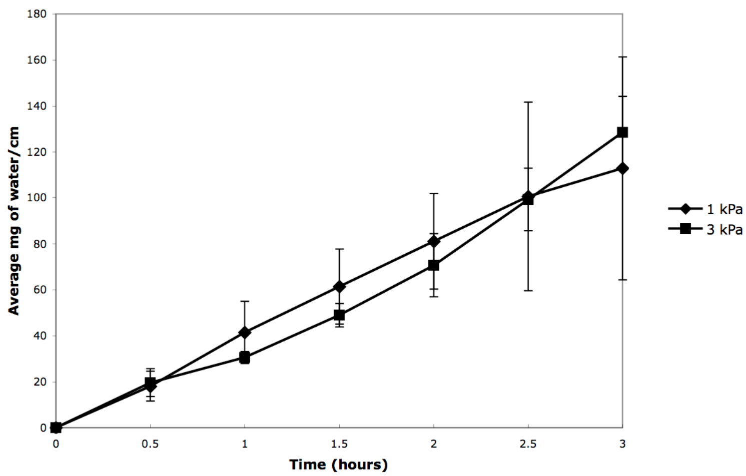

Figure 2. Water absorption measured at 1.0 kPa and 3.0 kPa in the spiral intestine.

In previous studies, we demonstrated the expression of acid mucins and a water-selective aquaporin (AQP4) in the *L. erinacea* spiral intestine [5,7]. From this data we hypothesized that the elasmobranch spiral intestine has the ability to absorb water. Our present findings support our hypothesis and further suggest that water absorption in the elasmobranch spiral intestine is likely facilitated by channel proteins such as aquaporins, which are specifically expressed in the intestine.

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1. **Aoki, M, Kaneko, T, Katoh, F, Hasegawa, S, Tsutsui, N and Katsumi, A.** Intestinal water absorption through aquaporin 1 expressed in the apical membrane of mucosal epithelial cells in sea-water adapted Japanese eel. *J of Exp Biol*. 206: 3495-3505, 2003.
2. **Evans, DH.** Osmotic and Ionic Regulation, In: Evans DH, editor. The physiology of fishes. Boca Raton, Florida: CRC Press, 1993.
3. **Evans, DH, Piermarini, PM, and Choe, KP.** The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev*. 85: 97-177, 2005.
4. **Reifel, CW and Travill, AA.** Structure and carbohydrate histochemistry of the intestine in ten teleostean species. *J Morphology*. 162(3): 343-360, 1979.
5. **Richards, EK, Simeone, A and Theodosiou, NA.** Water absorption in the spiral intestine of *Leucoraja erinacea*. *MDI Bulletin*. 48: 88-9, 2009.
6. **Roussel, P and Delmotte, P.** The Diversity of Epithelial Secreted Mucins. *Current Organic Chemistry*. 8: 431-437, 2004.
7. **Theodosiou, NA, Hall, D and Jowdry, A.** Comparison of acid mucin goblet cell distribution and *Hox13* expression patterns in the developing vertebrate digestive tract. *J Exp Zool (Mol Dev Evol)*. 308B: 442-453, 2007.
8. **Thorson, TB.** Partitioning of body fluids in the Lake Nicaragua shark and three marine sharks. *Science*. 138: 688-690, 1962.