Eggmosis: Effects of Egg Membrane Permeability on Egg Mass

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Abstract

Osmosis plays a crucial role in multiple aspects of our lives, from digestion to the way we respond to medications. Osmosis relies on the selectively permeable property of cell membranes, which allow certain ions and molecules to enter the cell while inhibiting the entry of others. We tested membrane permeability by exposing de-shelled eggs to different concentrations of corn syrup (mainly composed of glucose) using an isosmotic, hypoosmotic, and hyperosmotic solution. We weighed the eggs every 30 mins for 2 hours to generate the rate of change in g/min for each replicate. The mean rates of change in mass of eggs exposed to the isosmotic, hyperosmotic, and hypoosmotic solutions were 0.04g/min, -0.18g/min, and 0.090g/min respectively. A one-way ANOVA test and Post-hoc Tukey HSD test determined that the differences in change of rate in mass (g/min) between the eggs exposed to different treatments were statistically significant. Following two hours, the eggs exposed to the hyperosmotic solution were the smallest, while those exposed to the hypoosmotic solution were the largest. We, therefore, concluded that our hypothesis stating that the rate of change in mass (g/min) of each de-shelled egg will be significantly affected by the concentration of glucose solution to which they are exposed to was supported. In future studies, it is recommended that other molecules are used in the extracellular environment and that the trial times are extended in order to accurately determine the osmotic concentration inside the eggs.

Keywords: Osmosis, eggs, membrane permeability, cells

Introduction

The cell is the most basic unit of life, and every living organism on the planet is composed of at least one cell (Goodhead and Macmillan, 2017). Each cell is surrounded by a selectively permeable membrane which protects the inside of the cell from the extracellular environment (Anil Bajnath, 2021). As a result, some molecules enter the cell's membrane, such as nutrients, while others are blocked from entering, such as harmful toxins (Anil Bajnath, 2021). Additionally, a selectively permeable membrane allows water to travel down its osmotic gradient by osmosis. The difference in the number of particles in solution on each side of the membrane causes the formation of an osmotic pressure gradient, or the pressure necessary to stop water from flowing down its gradient, and the total number of particles in a solution

determines its osmolarity (Goodman and MacMillan, 2017). Research done on cell membrane permeability may be utilized to investigate cell membrane repair processes and plasma membrane integrity as well the effectiveness of nutrient absorption during digestion (Khoshbin & Camilleri, 2020; Dias and Nylandsted, 2021). Studying efficient nutrient absorption during digestion via membrane permeability can aid in the development of drugs that can more efficiently deliver nutrients to the body (Khoshbin & Camilleri, 2020; Dias and Nylandsted, 2021). To do this, researchers can measure the permeability of different membranes to various molecules, such as polysaccharides, proteins, lipids, and minerals (Khoshbin & Camilleri, 2020).

Underneath the hard shell of a *Gallus gallus domesticus* (hen's) egg, there is a membrane which is similar to that of mammalian cells in that it only allows the passage of certain molecules (Mittal et al., 2016). Therefore, the permeability of mammalian cells may be studied by comparing the permeability of hen egg membranes in various extracellular environments. This paper aims to demonstrate the rate of change in mass of a hen's egg when exposed to varying osmolarities of corn syrup (glucose), which cannot cross the egg's membrane due to its size, in the surrounding environment.

We hypothesize that since a de-shelled hen egg is selectively permeable to certain molecules and ions, the rate of change in mass of each de-shelled egg will significantly vary depending on the concentration of the glucose solution it is exposed to. We predict eggs in the hyperosmotic treatment will decrease in mass while those in the hypoosmotic treatment will increase in mass and the isosmotic group will not change. To test our hypothesis, we will expose de-shelled eggs to three solutions with varying concentrations of glucose, including a hypoosmotic, isosmotic, and hyperosmotic treatment. The isosmotic solution, which has the

same osmolarity of glucose as the egg's inside, will serve as our control. In comparison to the inside of the egg, the osmolarity of the hypoosmotic treatment will be lower while the osmolarity of the hyperosmotic solution will be higher.

Methods

To begin this experiment, we de-shelled nine white, medium eggs by placing them in a large bowl containing enough white vinegar to cover the eggs for 2 days. The bowl containing the eggs was covered with plastic wrap and safely stored at room temperature. They were periodically checked on to ensure that de-shelling did not take place earlier than expected. Once the eggs were deshelled, they were gently rinsed with water to remove any remaining residue and placed in a new bowl. The de-shelled eggs were stored in the refrigerator with a setting of 4°C until the day of the experiment. On the day of the experiment, three replicates were prepared for each of the isosmotic, hypoosmotic, and hyperosmotic treatments and labelled accordingly as “ISO, HYPO, and HYPER”.

In preparation for the isosmotic treatments, about 1.5 tbsp of glucose was added to each of the 500mL beakers labelled "ISO". Next, 250mL of water was added to each of the isosmotic beakers before adding a stir bar to each beaker. The stir bar was placed in each beaker to allow us to homogenize the liquids in each beaker so that they were all of the same consistency. To prepare the hyperosmotic solutions, 250mL of corn syrup was added into three 500mL hyperosmotic labelled beakers. The hypoosmotic was prepared by adding 250mL of water into each 500mL hypoosmotic labelled beaker.

Once all treatments and their replicates were prepared, three deshelled eggs were weighed and one was placed into each of the three “HYPER” labelled beakers. 1-2 drops of dye

were then added to each beaker for visual observation of the movement of water. All eggs for the treatments were observed and weighed in 30-minute intervals for a total of 2 hours. Once the weight of each egg was recorded, they were placed back in their respective beakers. Fifteen minutes after starting the timer for the hypertonic treatments, three new deshelled eggs were weighed and 1-2 drops of dye were added to each beaker. Once the weight of the three eggs was recorded, one egg was placed into each of the “ISO” labelled beakers. The eggs were taken out, gently wiped and weighed every 30 minutes. The eggs were then placed back into their respective beakers after their weights were recorded. Fifteen minutes after starting the timer for the isosmotic treatments, three new deshelled eggs were weighed and 1-2 drops of dye were added to each beaker. Once the weights of the three eggs were recorded, one egg was placed into each of the “HYPO” labelled beakers. The eggs were taken out, gently wiped and weighed every 30 minutes. The eggs were then placed back into their respective beakers after their weights were recorded.As seen in Figure 2, to ensure that all the eggs were completely submerged in the solution, a spoon was taped to the inside of each beaker to hold the eggs in place.

a.

b.

Figure 1a & 1b. 1a is the process of de-shelling eggs with vinegar. 1b shows our deshelled eggs ready to be used for the treatments.

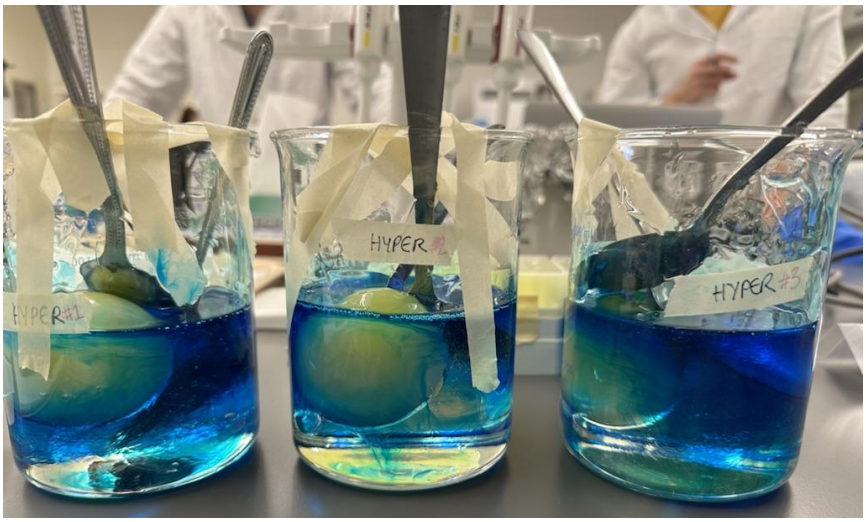

Figure 2. Three prepared replicates for the hyperosmotic treatment. The same was done for the isosmotic and hyperosmotic treatments. Spoons were used to hold down the eggs in the solution.

Figure 3. Process of weighing eggs every 30 minutes for each treatment.

Statistical Methods

The masses for each replicate were plotted on a graph every 30 mins to obtain the slope (rate of change in g/min). Using this data, a one-way ANOVA test was conducted with a significance level of $\alpha = 0.05$ on GraphPad Prism (version 9.3.1.471) to determine whether the mean rate of change of egg masses for each treatment type was significantly different from one another. The one-way ANOVA test showed that the mean values were significantly different with a p-value <0.0001. A Post-hoc Tukey HSD test was then performed on GraphPad Prism to determine which treatment groups specifically, (isosmotic, hyperosmotic, hypoosmotic) had significantly different means from one another. The test concluded that every treatment group's mean was significantly different from one another, with a p-value of at least < 0.05 between each group. Meaning that the rate of change of egg mass (g/min) was significantly different between all treatment types.

Results

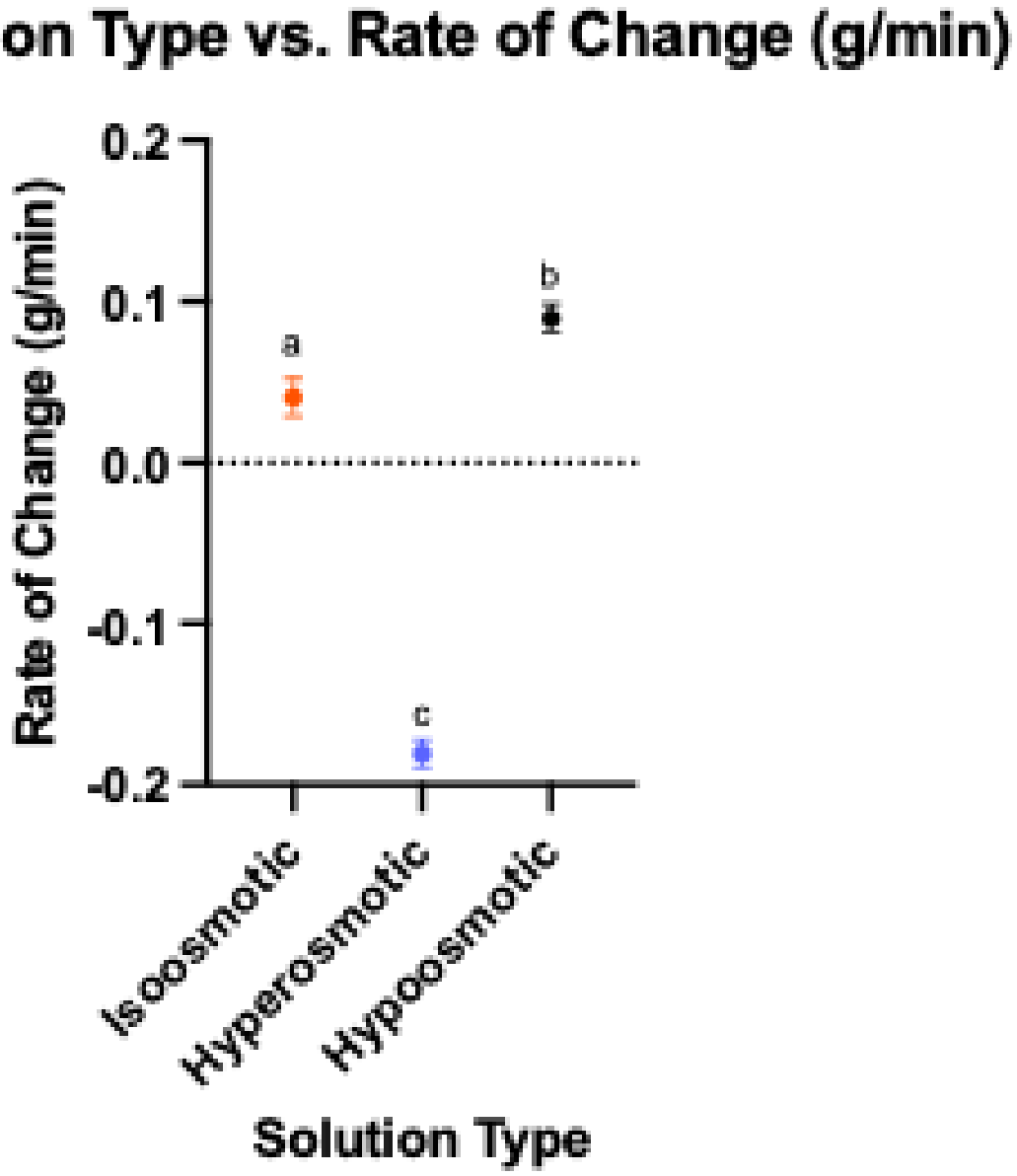

Figure 4. Rate of change in g/min of hen egg's mass after being exposed to an isosmotic, hypoosmotic or hyperosmotic solution. Each plot represents the mean rate of change (g/min) accompanied by a 95% confidence interval *(N=3)*. Different letters denote significant differences ($P<0.05$) between treatment types.

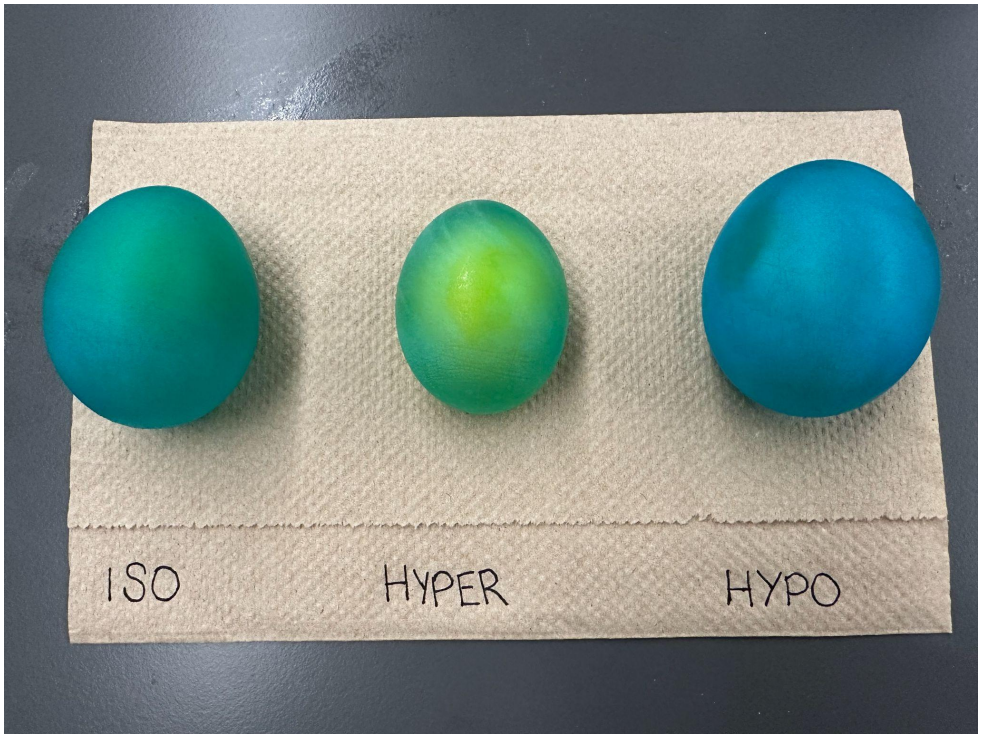

Figure 5. Visual comparison of ⅓ of the replicates for each treatment type [isosmotic (control), hyperosmotic, and hypoosmotic] after 2 hours.

Figure 4 shows the rate of change in g/min of the hen egg's mass after being exposed to an isosmotic, hypoosmotic or hyperosmotic solution. Medium white eggs were used for each treatment type, with a total of 3 replicates per treatment. The isosmotic solution (control) contained 250 mL of distilled water and 1.5 tablespoons of corn syrup. The hypoosmotic solution contained 250 mL of water only. The hyperosmotic solution contained 250 mL of corn syrup only. Each egg was weighed before being exposed to the solution and then every 30 mins for 2 hrs. The masses at every 30 mins for each replicate were plotted on a graph to obtain the slope (rate of change in g/min). Each plot represents the mean rates of change (g/min) accompanied by a 95% confidence interval. The mean rate of change in mass of eggs exposed to the isosmotic, hyperosmotic, and hypoosmotic solutions were 0.04g/min, -0.18g/min, and

0.090g/min respectively. A 1-way ANOVA and Post-hoc Tukey HSD multiple comparison tests showed that every treatment group was significantly different from one another.

Figure 5 shows a visual comparison of ⅓ of the replicates for each treatment type [isosmotic (control), hyperosmotic, and hypoosmotic] after 2 hours. Medium white eggs were used for each treatment type, with 3 replicates per treatment. The isosmotic solution (control) contained 250 mL of distilled water and 1.5 tablespoons of corn syrup, while the hypoosmotic solution contained 250 mL of water only, and the hyperosmotic solution 250 mL of corn syrup only. Additionally, 1 drop of blue dye was added to each solution for visual observation of water movement into the egg. The eggs exposed to the hypoosmotic solution were the largest in size at the end of the experiment, while the egg exposed to the hyperosmotic solution was the smallest. All eggs were stained by the blue dye from the outside regardless of whether water travelled into the egg.

Discussion

We hypothesized that due to a de-shelled hen egg's selective permeability to certain molecules and ions, the rate of change in mass of each de-shelled egg will vary depending on the concentration of the glucose solution (isosmotic, hyperosmotic, hypoosmotic) it is exposed to. The results we found were consistent with our initial predictions that the eggs exposed to the control isosmotic solution will show no rate of change in mass (g/min), while those exposed to the hypoosmotic and hyperosmotic solutions will show a positive and negative rate of change in mass (g/min) respectively. After performing a one-way ANOVA statistical test on GraphPad Prism we found that the average rate of change in egg mass (g/min) was significantly different between treatment groups with a p-value <0.0001. Additionally, a Post-hoc Tukey HSD test

indicated that every treatment group's mean rate of change in mass (g/min) was significantly different from one another, with a p-value of at least < 0.05 between each group. Accordingly, our hypothesis that the glucose concentration of the solution would affect the rate of change in egg mass (g/min) was supported by our results.

Our results can also be supported by biological processes from beginning to end. When an egg is submerged in vinegar for a day or two, the acetic acid in the vinegar reacts with the calcium carbonate crystals in the eggshell to produce carbon dioxide, exposing the soft membrane (Goodhead & MacMillan, 2017). What remains underneath the eggshell is described as a fibril and porous structure, which is composed of water-insoluble fibers arranged to form a semi-permeable membrane (Mittal et al., 2016). This semi-permeable membrane is key to osmosis, which is what we investigated in our egg experiment. A selectively permeable membrane is responsible for osmosis, which is the movement of water down its osmotic gradient (Vujovic, Chirillo, & Silverthorn, 2018). In our experiment, water was able to move across the egg membrane down its osmotic gradient while glucose was not due to its size. The isosmotic treatment contained an equal concentration of glucose as the inside of the egg. Therefore, there was no net movement of water into or out of the cell as there was no contraction gradient generated for it to use. Since the hypoosmotic treatment contained less glucose than the inside of the egg, water moved into the egg down its concentration gradient, increasing the egg's mass. Lastly, the hyperosmotic treatment contained a larger amount of glucose compared to the inside of the egg, so water showed a net movement out of the cell, decreasing the egg's mass over time. Similar results were seen by Goodhead and Macmillan (2017), who tested cell membrane permeability using mammalian red blood cells. Goodhead

and Macmillan (2017) demonstrate the effects of osmosis in blood cells by observing the changes in cell volume when placed in solutions of differing osmolarities and tonicities of NaCl. In fact, studying cell membrane properties can help explain multiple physiological processes that are biologically relevant to humans.

The data collected in this study regarding the permeability of the hen's egg membrane can be transferred to the behaviour of the mammalian plasma membrane due to their similarities in permeability. This data may be utilized to further investigate cell membrane repair mechanisms and plasma membrane integrity as described by Dias and Nylandsted (2021). In order to effectively respond to repeated damage sustained during cancer growth and invasion, cancer cells frequently overexpress proteins in the plasma membrane (Dias and Nylandsted 2021). It is generally acknowledged that enhanced membrane permeability during the early stages of cancer and many other disorders enables early detection and patient treatment (Dias and Nylandsted, 2021).

Additionally, absorption of nutrients from the food we ingest is possible due to the permeability of intestinal cells. The membrane provides fuel to cells by allowing nutrients into the cell and at the same time preventing certain molecules such as toxins from entering (Khoshbin & Camilleri, 2020). Different foods have different effects on the permeability of intestinal cell membrane permeability. For example, chocolate and alcohol increase permeability while fibers and vitamin D rich foods decrease permeability. High permeability can lead to toxins being transported into cells and the excretion of unwanted substances out of the cell, which may lead to many diseases such as colitis.

Limitations & Future Suggestions

Due to supply and time constraints, we only tested egg membrane permeability using corn syrup, which is primarily composed of glucose. To further advance the understanding of cell membrane permeability using de-shelled eggs, we recommend that future experimenters compare the rate of change in mass using different molecules, like sodium vs. glucose. Additionally, we recommend using more replicates for each treatment group and performing the experiment for a longer overall time. We were unable to reach an equilibrium (plateau) rate of change (g/min) for the eggs exposed to any of the solutions. Therefore, we predict that the eggs would have continued to lose and gain mass for more time. Our isosmotic solution, which served as the control treatment in our experiment, was designed to have the same osmolarity as the interior of an egg, but it was created by trial and error due to a lack of relevant literature on the intracellular osmolarity of an egg. Therefore, if the experiment is performed until an equilibrium is reached, the true osmolarity of the inside of an egg can be calculated and a more accurate control solution can be prepared. During the experiment, procedural difficulties were also experienced. For example, the eggs in the hyperosmotic solution floated up and were only half submerged in the corn syrup. We had to repeatedly push the egg's down with a spoon to ensure that they were exposed to the solution. Lastly, although we attempted to visually observe the water flow into the eggs using blue dye (figure 5), each egg was stained from the outside with the dye regardless of the solution it was exposed to. Thus, this was not the most reliable method for visualizing the amount of water travelling into the eggs.

Conclusion

Membrane permeability of de-shelled eggs to different concentrations of corn syrup (glucose) was tested by creating an isosmotic, hypoosmotic, and hyperosmotic solution using the corn syrup. The masses at every 30 mins for each of the 3 replicates per treatment were plotted on a graph to obtain the slope (rate of change in g/min). Based on a one-way ANOVA and a post-hoc Tukey HSD multiple comparison test, every treatment group differed significantly from one another, with a p-value <0.05 between each treatment group. Therefore, our hypothesis stating that there would be a difference in the rate of change of egg mass when they are exposed to varying concentrations of glucose was supported. Further research using de-shelled eggs to study cell membrane permeability should compare the rate of change in egg mass after exposure to various concentrations of different impermeable molecules (ie., comparison between sodium and glucose).

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