California State University, Northridge

INVOLVEMENT OF L (-) RHAMNOSE IN SEA URCHIN GASTRULATION: A
LIVE EMBRYO ASSAY

A thesis in partial fulfillment of the requirements
For the degree of Master of Science
in Biology

By

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Abstract

INVOLVEMENT OF L (-) RHAMNOSE IN SEA URCHIN GASTRULATION: A LIVE EMBRYO ASSAY

By,

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Master of Science in Biology

The sea urchin embryo is a National Institutes of Health model system that has provided major developments later found of importance in human health and disease. To obtain initial clues to identify glycans that mediate cellular interactions, *Lytechinus pictus* sea urchin embryos were incubated at 24 and 30 hrs post-fertilization with 0.0009M – 0.03M alpha cyclodextrin, melibiose, L(-) rhamnose, trehalose, D(+) xylose and L(-) xylose in lower calcium artificial sea water (pH 8.0, 15°C) that speeds entry of molecules into the interior of the embryos. While alpha cyclodextrin killed the embryos, and L (-) xylose had small effects at one concentration, L (-) rhamnose statistically caused increased numbers of unattached archenterons and exogastrulated embryos at low glycan concentrations after 18-24 hrs incubation with the sugar. The results were statistically significant compared to control embryos in the absence of sugar ($p < 0.05$). The other sugars melibiose, trehalose, D (+) xylose had no statistically significant effects whatsoever at any concentration. A total of 39,369 embryos were examined in this study. Other studies have identified L (-) rhamnose and L (-) rhamnose- binding receptors in cellular interactions. This is the first demonstration of a likely role for L (-) rhamnose in

cellular interactions using a live embryo assay in the NIH designated model sea urchin embryo system.

I. Introduction

Important components and processes that drive early embryogenesis include cell adhesion mechanisms, cell receptors and cell signaling molecules, and cell motility. They are vital in the regulation of embryonic cell development, the determination of cell fate, and communication amongst cells. Inhibition of cell-cell adhesion and cell signaling can hinder the development of an organism. This study explores carbohydrates that could potentially have an effect on embryonic development by inhibiting vital developmental processes. The aim of this investigation is to discover the possible carbohydrate effect on sea urchin embryos at early gastrulation phase in hopes of shedding light on the fields of developmental and cancer biology. Understanding cell communication and adherence processes may lead to an improved comprehension of human disease mechanisms.

Cells communicate via cell-surface receptors. Some of which are proteins; others are carbohydrates and/or glycoproteins. Cell surface carbohydrates are important in adhesion mechanisms (Sharon and Lis, 1986). The mechanisms of adhesion are not completely understood. The adhesion strength is often strongest between that of a normal cell and a cancer cell, and weakest between two cancer cells (hence the cancer cells' ability to invade and be mobile). Studies have shown that when cells are grown *in vitro* in media that lacked calcium, the cells disaggregated (Khurram et al., 2004, Humphreys, 1963). Other studies have demonstrated that cancer cells contain half the amount of calcium than normal cells (Durham and Walton, 1982). This provides evidence that low calcium concentrations are important for altered cancer cell adhesions (Durham and Walton, 1982);

therefore, media containing low calcium concentrations are important when conducting research involving embryonic cell adhesion mechanisms.

The Sea Urchin

The sea urchin is used as an important resource in studying embryonic cellular environments and embryonic cellular activities. It is valuable in that its gametes are easily accessible and plentiful. The urchin is fecund, and its embryos are large, transparent, and can be manipulated fairly easily. In addition, the first stages of the sea urchin life in embryo form are similar to that in human embryos. The National Institutes of Health (NIH) has designated the sea urchin as a model system due to the numerous basic cellular physiological processes that have been discovered in sea urchin studies and later found important in human disease like cancer (Cameron et al., 2000). The following developmental phases are processes in which sea urchins and humans have in common.

During the process of fertilization, the sperm's contact with the outer layer of the oocyte causes the acrosome reaction, in which the sperm releases enzymes that allow the sperm to get through the ovum's outermost layers: the jelly coat and the vitelline layer (Osman et al., 1989, Tosti and Boni, 2004). This reaction allows for the plasma membrane of the sperm to fuse and become continuous with the plasma membrane of the ovum (Osman et al., 1989). This contact triggers a series of cellular events. First is the cortical reaction, where the cortical granules (located just beneath the egg plasma membrane surface) release enzymes that alter sperm receptors present on the surface of the vitelline layer (Gardner et al., 2007), a

process vital in preventing polyspermy (Gardner et al., 2007). Another mechanism to prevent polyspermy occurs one second post-sperm/egg contact; an influx of sodium ions causes the membrane of the ovum to depolarize (Tosti and Boni, 2004). The vitelline layer is disconnected from the plasma membrane and the newly visible membrane is important in blocking polyspermy as well (Tosti and Boni, 2004). The hyaline layer is derived from the vitelline layer and is important in keeping the embryo together during cell cleavage throughout the early phases of development (Tosti and Boni, 2004).

The sea urchin undergoes radial holoblastic cleavage, where the first and second cleavages are vertical and the third cleavage is equatorial; each of these first three cleavages produce cells of equal size (Drasdo and Forgacs, 2000; Davidson et al., 2008) forming animal and vegetal hemispheres. The fourth cleavage is different from the preceding cleavages. During this cleavage, the four cells of the animal region divide vertically and are of equal size (Drasdo and Forgacs, 2000). These cells are considered to be mesomeres and are medium in size. The cell cleavage of the vegetal hemisphere is horizontal and results in unequal cleavage producing four large cells, macromeres, and four small cells, micromeres, which are located just below the macromeres (Drasdo and Forgacs, 2000; Davidson, et al., 2008). The four mesomeres eventually develop into the ectoderm; the four macromeres develop into some ectodermal components and the entire endoderm, and the micromeres become primary mesenchymal cells that form structural components of the embryo (Davidson et al., 2008).

In humans and sea urchins, development progresses from the morula stage, to blastula, and gastrula stage. The morula is defined as an early developmental stage of an embryo where the blastomeres form a compact (solid) mass of about 16 to 32 cells, as a result of repeated mitotic divisions of a fertilized egg (Davidson et al., 2008). The morula then develops into the blastula.

Blastulation

The Blastula is the stage within the series of early stages of development where the embryo is a hollow ball of cells. During this phase, the hyaline layer is pushed outward to hollow out the embryo forming the blastocoel located in the central portion of the embryo (Fink and McClay, 1985; Carroll et al., 2008). In addition, the osmotic pressure causes the ingression of the primary mesenchyme cells leading to invagination at the vegetal pole of the embryo (Fink and McClay, 1985).

Gastrulation

The invagination of the vegetal plate forms the archenteron, and the blastopore of the embryo (Fink and McClay, 1985). As the cells continue moving inward, the archenteron elongates causing it to move across the center of the embryo towards the animal pole region (Fink and McClay, 1985) (See Figure 1). During this phase, not only does the archenteron continue to elongate and move across the blastocoel portion of the embryo, but it also attaches to the animal region of the embryo proper (Fink and McClay, 1985).

Gastrulation is an important phase of developmental biology where the full body plan of the organism is established with generation of the primary germ layers. The cells of the archenteron begin to lose affinity for the hyaline layer and for its surrounding cells, and begin to gain affinity for the basal lamina (Hardin and Cheng, 1985). As the archenteron elongation continues, the cells of the archenteron tip are secondary mesenchymal cells (Hardin and Cheng, 1985). These cells develop extensions called filopodia which aid the elongation of the archenteron by exhibiting a contractile motion as well as adhering to the basal lamina (Hardin and Cheng, 1985); eventually allowing for the attachment of the archenteron to the blastocoel roof (Hardin and Cheng, 1985). Cell motility and cell adhesiveness are important mechanisms of gastrulation because without the archenteron attachment to the blastocoel roof, the organism will not progress to the next stage of embryogenesis (Hardin and Cheng, 1985).

Carbohydrates

There are four different classes of organic molecules: proteins, nucleic acids, lipids and carbohydrates (Ghazarian et al., 2010). Out of the four principal biological molecules, carbohydrates are most abundantly found in nature and are synthesized and metabolized by almost every organism (Ghazarian et al., 2010). Despite the abundance of carbohydrates, their physiological role in adhesion, cell-cell communication and motility is poorly understood.

Carbohydrates have a general empirical formula of $C_nH_{2n}O_n$ and are result of water and carbon combining during photosynthesis and combining with other

macromolecules to form complex molecules. Carbohydrates form long chains that extend off the cell surfaces. Because of this feature, they are involved in the initial interaction with other cells and/or substrates (Oppenheimer et al., 2008).

Glycoproteins are proteins with covalently bound carbohydrates and are important in intercellular interactions and cell substrate interactions (Maeder, 2002). Because of their ability to form extended chains, they are able to participate in biological processes such as cell adhesion and cell-cell communication (See Figure 3); however, some of the mechanisms necessary to carry out these biological functions are unknown (Maeder, 2002).

The diversity of glycan, or polysaccharide-linked protein is dependent on enzyme activity of glycosyl transferases and glycosidases which play a role in the secretory pathway of the glycans (Dube and Bertozz, 2005). N-linked glycosylations involve conjugations glycans to an asparagine of the carrier proteins (Dube and Bertozz, 2005). According to a study by Herbert (2005), this category of glycans plays an important role in signaling and recruitment of chaperone proteins, and is a means of quality control in sorting receptors in the lumen of the endoplasmic reticulum in the secretory pathway. Complex N-linked glycans are built up and remodeled in the endoplasmic reticulum and Golgi, and include the constituent sugars N-acetyl galactosamine, galactose, neuraminic acid, N-acetylglucosamine, fructose, mannose, and fucose.

O-linked glycosylation results from glycans being attached to the hydroxyl group of threonine or serine residues of a carrier protein. Examples of O-linked glycans include the constituent sugars glucosamine, xylose, galactose, fucose, and mannose. O-linked

glycosylated proteins are abundant in the zona pelucida of mammalian oocytes and serve as receptors for sperm (Hokke, 2005). They also play a role in immunological processes/mechanisms in mammals (Tsuboi and Fukuda, 2000), involving inflammatory responses, ABO blood antigens, and hematopoiesis (Tsuboi and Fukuda, 2000, Hokke, 2005).

Lectins are proteins or glycoproteins that bind carbohydrates (Goldstein and Hayes, 1978) and are found in plants and animals (and Hayes, 1978; Barthel, 2007). Plant lectins have been very useful in the investigation of the functional significance of carbohydrates in cell surface receptors on tumors, normal cells, and embryonic cells Krach et al. (1974) because they allow a focus on the changes that occur in sea urchin embryo cells' surfaces. One such study involved in the observation of agglutination of developing sea urchin embryos with plant-derived and was found that some alterations occurred in the carbohydrate-containing cell surface sites during differentiation and maturation (Krach et al., 1974). The carbohydrates that are on cell surfaces are thought to contain information that controls morphogenesis due to the fact that they appear to modulate the initial contact with other cells and cell substrates. It is also known that some carbohydrates are also linked with tumors.

Changes in glycosylation not only include over and under expression of naturally occurring carbohydrates/glycans but also newly expressed glycans that are specific to embryonic cells (Dube, 2005). These changes are due to changes in enzyme levels in the Golgi apparatus and affect the core structure of the either N-

linked and O-linked proteoglycans causing alterations of the terminal structure that is correlated with malignancy (Dube, 2005).

In efforts to understand the mechanisms of carbohydrate involvement in gastrulation (Latham et al., 1999) suggested the mechanism for how some carbohydrates may interfere with the binding of the archenteron tip to the blastocoel roof. This article showed that both *Lens culinaris* and *Pisum sativum* lectins that bind mannose/glucose caused exogastrulation due to D-mannose/glucose-like residues, perhaps playing a role in archenteron development (Latham et al., 1999) (See figure 2). The authors further suggest that wheat germ agglutinin (WGA) bound to mesenchymal cells were involved in skeletogenesis of the embryo and caused defective skeletogenesis (Latham et al., 1999).

Another study showed that tunicamycin and sodium selenate along with glycosidases beta-amylase, alpha-glucosidase, and alpha-mannosidase block archenteron organization, elongation, and subsequent attachment to the blastocoel roof (Khurruum et al., 2004). These results suggested that there is carbohydrate involvement in the cell-cell interactions in sea urchin gastrulation. In the absence of these inhibitors, the archenterons attached normally. In the presence of these molecules, exogastrulation occurred coupled with the effects of archenteron disorganization and lack of archenteron elongation (Khurruum, et al., 2004). This work suggested a mechanism by which *Lens culinaris* lectins interact with the archenteron cells' adhesion to the blastocoel roof (Khurruum et al., 2004). This research suggests that *Lens culinaris* lectins enter the gastrula and binds to the D-

mannose-like residues on the archenteron tip and/or on the blastocoel roof, blocking the normal adhesion interaction and causing exogastrulation (Khurram et al., 2004).

Cell surface carbohydrates are vital components in sea urchin gastrulation (Sajadi, et al., 2007). Tunicamycin is an antibiotic that inhibits the first step in the synthesis of lipid-linked oligosaccharides that function as intermediates in the biosynthesis of N-linked glycoproteins (Sajadi et al., 2007). The drug also causes changes in cell morphologies in cell cultures, alterations in adhesion, and cell recognition and decreases cell surface receptor binding (Sajadi et al., 2007). During gastrulation of the sea urchin embryo, tunicamycin has been shown to block development (Heifetz and Lennarz, 1979)

Processes such as cell growth, motility, and cell recognition are controlled by cell surface interactions (Roth and Weston, 1967; Oppenheimer, 1979). Studies have shown that cell interactions are mediated by cell surface carbohydrates that bind to receptor sites that may be specific to enzymes or lectins (Oppenheimer, 1979). Secondary mesenchymal cells of sea urchins (during the gastrula phase of development) did not have normal binding in the presence of D-galactose and N-acetyl-D-galactosamine; these sugars inhibited the binding and hence the attachment of the archenteron tip to the blastocoel roof (Oppenheimer et al., 1979).

Carbohydrates and lectins play a critical role in cell-cell communication, adhesion, and cell motility. In this study, I investigate the effects of six different carbohydrates on *Lytechinus pictus* embryos at the late blastula and gastrula stages. The six carbohydrates used in this study were: α -cyclodextrin, melibiose, L (-) rhamnose, trehalose, D (+) xylose, and L (-) xylose. The objective was to observe

the carbohydrates' effect on the embryos and see what morphological outcomes are produced. If some carbohydrates influence sea urchin development, this could provide a clue as to the receptors involved in mediating specific cellular interactions in the sea urchin embryo system.

II. Materials and Methods

Solution Preparation.

Artificial seawater preparations (g/L of distilled water): artificial seawater was prepared by placing four liters of distilled water in a large four liter Erlenmeyer flask. Each of the following reagents (all from Sigma-Aldrich, St. Louis MO) was added slowly and allowed to mix for 15 minutes: sodium chloride (24.72M), potassium chloride (0.67M), calcium chloride dihydrate (1.36M), magnesium chloride hexahydrate (4.66M), magnesium sulfate heptahydrate (6.29M). After 15 minutes of stirring had elapsed, sodium bicarbonate (0.18M) was mixed into solution. The solution was allowed to mix for 20 more minutes or until the contents were completely dissolved. The pH was checked, using Accumet model 5 pH meter (Fisher Scientific, Hanover Park, CA, USA), to insure that it was 8.0. In the event that the pH was lower than 8.0, small amounts of Trizma base (Sigma-Aldrich, St. Louis, MO, USA) were added or if it was too high, small amounts of Trizma acid (hydrochloride) (Sigma-Aldrich, St. Louis, MO, USA) were added to solution.

1.5mM low calcium sea water preparations: low calcium seawater was prepared using two liters of distilled water in a four liter Erlenmeyer flask. The following reagents were added to the water (all from Sigma-Aldrich): sodium chloride (24.72M), potassium chloride (0.67M), calcium chloride dehydrate (0.113M), magnesium chloride hexahydrate (4.66M), magnesium sulfate heptahydrate (6.29M). After 15 minutes of stirring lapsed, sodium bicarbonate (0.18M) (Sigma-Aldrich, St. Louis, MO, USA) was

mixed into solution. The pH of the solution was checked and adjusted as described previously.

Potassium Chloride Solution

A 0.55M potassium chloride solution was prepared by adding 8.2 g of potassium chloride (Sigma-Aldrich, St. Louis, MO, USA) to 200 mL of water and allowed to mix until the potassium chloride was dissolved. The final concentration was 0.55M.

Fertilization: Sea Urchin Gametes and Fertilization

Lytechinus pictus sea urchins purchased from Marinus Inc., (Garden Grove, CA, USA) were selected from the aquarium. The goal was to select one male and 15 one female. Urchins were selected via the coloration appearance around the mouth of the urchin; females tend to be brown or pink and males tend to be white to clear around the mouth of the urchin. The sea urchins were collected, and kept on damp paper towels. In order to cause the release of gametes, 3 mL of 0.55M potassium chloride solution was injected intracoelomically via a 27.5 gauge needle and a 1 mL syringe around the mouth of the sea urchin. The potassium chloride was injected slowly over a period of one to two minutes per sea urchin. After the injection step was complete for one urchin, the syringes were rinsed between sea urchins. The female urchins released eggs that were brown in color and the males secreted sperm that was white in color.

To collect the eggs, a small 50 mL beaker (a diameter approximating that of the urchin) was filled with 40 mL of artificial seawater. The female urchin was placed gonoduct surface down on the beaker that was covered with parafilm; a small hole was

cut into the Parafilm so that the sea urchin could sit atop the beaker allowing the eggs to be secreted into the water of the beaker without falling into the beaker. Once the eggs were completely settled to the bottom of the beaker, they were washed by aspirating off the excess supernatant. Then the eggs were transferred through a 165 μ m mesh sheet to filter the eggs into a new 50 mL beaker containing fresh artificial sea water followed by an additional wash of 100 mL of artificial sea water. Then the eggs were allowed to settle for 20 minutes. This washing step was repeated three times. The shape of the eggs was examined under a microscope to help ensure that they were healthy and viable.

The sperm was prepared by placing the urchin gonoduct surface down on a 35x10 mm Petri dish on ice. Once 1 mL of sperm was accumulated, 1 mL of sperm was diluted in 10 mL of artificial seawater.

Fertilization

The fertilization of eggs by sperm was tested by placing a small drop of the egg suspension onto a slide, followed by a small drop of sperm suspension. The egg/sperm mix was observed using a light microscope at 100x magnification. If the sperm caused formation of the fertilization membrane on the eggs, then the remainder of the eggs were fertilized: one mL of the diluted sperm was transferred to the same dish as the eggs and time was recorded. The solution was gently mixed, and the embryos were allowed to settle. Once the embryos settled, they were again washed by adding artificial seawater, allowing them to settle once again. The embryos were washed three times as described previously, then transferred into a larger casserole dish and allowed to sit for 30 minutes at room temperature before being placed into a 15°C incubator.

Carbohydrate Preparations

There were six carbohydrates used in this study; each of them was used at concentrations of 0.03M, 0.0015M, 0.003M, 0.0015M, and 0.0009M. The carbohydrates were prepared by diluting a specified amount of the carbohydrate into 10 mL of low calcium artificial sea water. The pH of each of the solutions was checked to ensure a pH solution of 8.0. Table 1 refers to the specific amounts of carbohydrates diluted for each of the desired concentrations.

Sugar	0.03M	0.015M	0.003M	0.0015M	0.0009M
D (-) Xylose	0.046 g	0.022 g	0.006 g	0.002 g	0.0014 g
L (+) Xylose	0.044 g	0.022 g	0.006 g	0.002 g	0.0014 g
L (-) Rhamnose	0.054 g	0.028 g	0.006 g	0.004 g	0.0016 g
Melibiose	0.102 g	0.054 g	0.014 g	0.006 g	0.004 g
Trehalose	0.114 g	0.056 g	0.014 g	0.008 g	0.004 g
α-Cyclodextrin	0.291 g	0.146 g	0.036 g	0.018 g	0.008 g

Table 1

Table 1: Carbohydrate Dilution Preparation

The table provides the recipe for each carbohydrate that was measured and then added to the low calcium sea water. In order to prepare the concentration treatments, a specified amount of each carbohydrate was measured and diluted with 10 mLs of low calcium sea water.

The carbohydrate treatments were distributed (50 μ L per well) among rows in a 96 well plate. The carbohydrates used in this experiment were purchased from Sigma-

Aldrich, St. Louis, MO, USA, and the catalogue numbers are as follows: α -cyclodextrin (C4642), melibiose (M5500), trehalose (T5251), D (+) xylose (X3877), L (-) xylose (X1625), and L (-) rhamnose (R3875).

Carbohydrate treatments

Embryo viability was first checked by light microscopy at 100x magnification. If the embryos were alive and moving, then they were deemed as viable and ready for carbohydrate treatment.

At 24 hours post-fertilization, 25 μ L of the embryo suspension was transferred to each of the wells in a 96 well plate. The first row was designated for the control embryos (25 μ L of low calcium artificial seawater plus 25 μ L embryo suspension). Following the control row, the plate was set-up as follows: 3 rows of wells were designated for the highest concentration 0.03M, and next 3 rows were designated for the next lowest and so forth. A total of four 96 microwell plates were used for each of the sugars: two for 24 hours post-fertilization and two for 30 hours post-fertilization; each well consisting of 10 to 30 embryos in 25 μ L plus 50 μ L of the carbohydrate mixture. The microwell plates were placed in the 15°C incubator overnight.

Day 3: Fixing the Embryos and Counting

10% formaldehyde solution preparation: 3 μ L of 37% formaldehyde was added to 7 μ L of artificial seawater and mixed well. Fixing the embryos and counting: at 48 hours post-fertilization, all 24 hours and 30 hours post-fertilization treated plates were fixed by

adding 10 μ L of 10% formaldehyde solution to each well, making the total volume of the wells 85 μ L.

Embryos were counted under a microscope and classified as possessing one of the following morphologies: Completely attached archenteron (CA), Unattached archenteron (UA), Exogastrulation (EXO), Non-viable (NV), and Non-Invaginated (NINVG).

Statistical Analysis

Each of the morphologies were tallied and combined to give a total population size. Percentages of the resulting morphologies were calculated, along with standard deviations and standard error. The results were analyzed using an ANOVA followed by Tukey Pairwise Comparison test calculated using SYSTAT 13 software.

III. Results

In this study, six different sugars were used as treatments of late blastula stage and early gastrula stage *Lytechinus pictus* embryos as means of testing their effect during the gastrulation phase of development. During gastrulation, the major event occurring is the primitive gut formation. The formation of the primitive gut is determined by the attachment of the archenteron to the blastocoel roof. In this experiment carbohydrates that were used included α -cyclodextrin, melibiose, L (-) rhamnose, trehalose, D (+) xylose, and L (-) xylose. Each of the carbohydrates was used at concentrations of: 0.03M, 0.015M, 0.003M, 0.0015M, and 0.0009M. The control embryos were treated solely with low calcium artificial sea water. The possible resulting morphologies of the different treatments were as follows: complete archenteron attachment (CA), unattached archenteron (UA), exogastrulation (EXO), non-viable (NV), and no invagination (NINVAG). Each carbohydrate treatment was added either at 24 hours post-fertilization or 30 hours post-fertilization. The embryos were later fixed using 10% formaldehyde at 48 hours post-fertilization, and the effects were observed and counted. An ANOVA was run followed by a Tukey Pairwise Comparison to compare each of the concentration treatments to the control population.

Control Population

The control population was treated with low calcium artificial sea water at 24 or 30 hours post-fertilization. The embryos displayed about 99.8% complete

attached archenteron. A total of 3,165 control embryos were used throughout the entire experiment, and 3,159 had complete attached archenterons.

α -Cyclodextrin treatments

α -cyclodextrin was added at 24 hours post-fertilization and development allowed to proceed until fixation at 48 hours (Figures 4, 5, and 6A-6F and Tables 2 and 3). A total of 5,343 embryos were used with the 24 hour post-fertilization group treated with α -cyclodextrin, 437 of them were control embryos. Concentrations 0.03M and 0.015M α -cyclodextrin had a statistically significant effect ($p < 0.05$), resulting in the production of more non-viable embryos.

When α -cyclodextrin was added at 30 hours incubation as above (Figures 7, 8, 9A-9F and Tables 4 and 5) the same two concentrations of α -cyclodextrin had a statistically significant effect on the embryos ($p < 0.05$). These concentrations produced more non-viable embryos when compared to the control population. In experiments initiated at both 24 and 30 hours, the control embryos (seawater only) possessed 100% complete archenteron attachment. A total of 2,817 embryos were treated with α -cyclodextrin 30 hours post fertilization, 307 of them were control embryos.

Melibiose treatments

When melibiose was added at 24 hours incubation, the following results were obtained (Figures 10, 11, 12A-12F and Tables 6 and 7) at 48 hours. The melibiose treatments 24 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 3,784

embryos were treated with melibiose, 300 of them were used as the control population. The control population had 99% complete archenteron attachment.

When melibiose was added at 30 hours incubation, the following results were obtained (Figures 13, 14, and 15A-15F, Tables 8 and 9) at 48 hours. The melibiose treatments 30 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 4,071 embryos were treated with melibiose, 285 of them were used as the control population. The control population had 100% complete archenteron attachment.

L (-) Rhamnose treatments

When L (-) rhamnose was added at 24 hours incubation, the following results were obtained (Figures 16, 17, 18A-18B, Tables 10 and 11) at 48 hours. L (-) Rhamnose 24 hours post-fertilization had a significant effect at concentrations: 0.03M, 0.015M and 0.0015M. Concentrations 0.03M and 0.015M statistically produced more unattached archenterons, while 0.0015M produced more embryos with exogastrulation. The resulting p-values were less than 0.05 for the three concentrations. The control population had 96% complete archenteron attachment. There were 2,313 embryos used with this carbohydrate treatments, 172 of them were used as control embryos.

When L (-) rhamnose was added at 30 hours incubation, the following results were obtained (Figures 19, 20, 21A-21F, Tables 12 and 13) at 48 hours. Concentration 0.003M, 0.0015M and 0.0009M had a significant effect on the embryos. These three treatments produced more exogastrulation when compared to

the control population. The resulting p-values for the three statistically significant concentrations were less than 0.05. There were 3,820 embryos used for the L (-) Rhamnose 30 hours post-fertilization treatments, 270 of them were used as control embryos.

Trehalose treatments

When trehalose was added at 24 hours incubation, the following results were obtained (Figures 22, 23, 24A-24F, Tables 14 and 15) at 48 hours. The trehalose treatments 24 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 1,810 embryos were treated with trehalose 24 hours post-fertilization, 146 of them were used as the control population.

When trehalose was added at 30 hours incubation, the following results were obtained (Figures 25, 26, 27A-27F, Tables 16 and 17) at 48 hours. The trehalose treatments 30 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 4,071 embryos were treated with trehalose 30 hours post-fertilization, 285 of them were used as the control population. The control population had 100% complete archenteron attachment.

D (+) Xylose treatments

When D (+) xylose was added at 24 hours incubation, the following results were obtained (Figures 28, 29, 30A-30F, Tables 18 and 19) at 48 hours. The D (+)

xylose treatments 24 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 3,590 embryos were treated with D (+) xylose 24 hours post-fertilization, 210 of them were used as the control population.

When D (+) xylose was added at 30 hours incubation, the following results were obtained (Figures 31, 32, 33A-33F, Tables 20 and 21) at 48 hours. The D (+) xylose treatments 30 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 3,995 embryos were treated with D (+) xylose 30 hours post-fertilization, 216 of them were used as the control population. The control population had 100% complete archenteron attachment.

L (-) Xylose treatments

When L(-) xylose was added at 24 hours incubation, the following results were obtained (Figures 34, 35, 36A-36F, Tables 22 and 23) at 48 hours. The L (-) xylose treatments 24 hours post-fertilization did not have an effect on the embryos. After analyzing the data, all of the p-values were greater than 0.05. A total of 4,358 embryos were treated with L (-) xylose 24 hours post-fertilization, 444 of them was used as the control population.

L(-) xylose was added at 30 hours incubation the following results were obtained (Figures 37, 38, 39A-39F, Tables 24 and 25) at 48 hours. Concentration 0.003M had a significant effect on the embryos. The treatment produced more unattached archenterons when compared to the control population. The resulting p-

value the statistically significant concentration was less than 0.05. There were 1,520 embryos used for the L (-) Rhamnose 30 hours post-fertilization treatments, 148 of them were used as control embryos.

IV. Discussion

In this study, the goal was to observe the effects of carbohydrates on the cellular interactions and cell adhesiveness among sea urchin embryos. It has been noted from previous studies that carbohydrates play a role in cellular binding. In fact, carbohydrates are thought to be the initial contact in cell-cell and/or cell- substrate binding processes in that carbohydrates tend to be extensions of the cell plasma membrane.

In studying carbohydrate effects on cellular interactions in sea urchin embryos, sea urchin gastrulas were introduced to specific concentrations of carbohydrates and specific cellular interactions were observed. Six carbohydrates were used in the investigation: α -cyclodextrin, melibiose, L (-) rhamnose, trehalose, D (+) xylose and L (-) xylose, and the dilutions selected were based on another study that used similar biological concentration ranges (Le, 2008). It was decided to see which concentrations of these carbohydrates are most effective, and to study the effects at two time points. In normal development the primary mesenchymal cells are beginning to aggregate and ingress at 24 hours post-fertilization, and a primitive archenteron is somewhat established at the vegetal pole. At 30 hours, it is expected that the archenteron has formed and will be about mid-way in the blastocoel, making its way to the animal pole. 30 hours was selected as a time point of treatment in this study because the archenteron is already formed at this point, and it was important to determine if the carbohydrates had an effect on the mobility to the animal region. If any were effective in inhibiting the cell binding, then exogastrulation or unattached archenterons might result. At 48 hours, when the embryos

were fixed, the archenteron should be completely attached to the blastocoel roof, in the animal region of the embryo.

Monosaccharides

Three monosaccharides were used in this study: D (+) xylose, L (-) xylose, and L (-) rhamnose. Interestingly, out of the three, L (-) rhamnose had the largest effect and statistically significant effect on both 24 and 30 hour post-fertilization treated embryos. The other two also, showed difference from the controls.

L (-) Rhamnose

L (-) Rhamnose produced more exogastrulation and unattached archenterons than the control populations. This carbohydrate appeared to inhibit the cellular adhesion interactions, and hence attachment of the archenteron to the blastocoel roof. The L (-) rhamnose treatment was not time dependent since they had an effect on the embryos at both 24 and 30 hours post-fertilization. However, the resulting morphologies differed in that more unattached archenterons were produced with the 24 hour post-fertilization treatments. Furthermore, both time points produced some exogastrulation. L (-) rhamnose is effective possibly because it possesses the capability to bind to lectins on either the filopodia portion of the archenteron tip (the secondary mesenchymal cells) or on cells of the blastocoel roof; in general, L(-) rhamnose is able to interfere with the cellular interactions of archenteron attachment to the blastocoel roof. But because this assay on live whole embryos is indirect, the site of action of the carbohydrates cannot be precisely identified.

Other studies have investigated rhamnose and its role in cell-cell adherence. An investigation studying rhamnose-binding lectins and their role in host-pathogen interactions specifically aimed to investigate the mechanism pathogens use to invade their host (Watanabe, 2008). As part of the study, they used an assay that measured rabbit erythrocyte agglutination in the presence specified sugars, one of the sugars investigated was rhamnose (Watanabe, 2008). Results showed rhamnose to be most effective in inhibiting cell adherence because at very minimal concentrations, it produced the least amount of erythrocyte agglutination (Watanabe, 2008). The study further suggests rhamnose-binding lectins are important in immunological response in regards to invading pathogens (Watanabe, 2008).

A study was performed to observe the inhibition of cancer cell growth and metastasis with the use of modified pectins (Nangia-Makker, et al., 2002). Pectins are complex carbohydrates that serve a role in cell-cell adhesion in plant cells (<http://www.cancer.org/Treatment/TreatmentsandSideEffects/ComplementaryandAlternativeMedicine/DietandNutrition/modified-citrus-pectin>). The experiment was performed by injecting nude mice with breast carcinoma cells and then treating them with dietary modified citrus pectin (MCP) in their drinking water (Nangia-Makker, et al., 2002). When analyzed, it was found that the MCP was rich in galactose, L (-) rhamnose, and xylose (Nangia-Makker, et al., 2002); it was also found that the MCP was effective in its ability to bind and inhibit recombinant galectin-3 and recombinant galectin-3 cellular function, such as tumor cell aggregation, binding of tumor endothelial cells, anchorage independent growth, and

binding to the laminin ligand (Nangia-Makker, et al., 2002). MCP was able to interfere with these cellular activities via binding to the carbohydrate region of the galectin-3 (Nangia-Makker, et al., 2002). Although L (-) rhamnose was not the sole carbohydrate present in the lectin of interest, this investigation is another example of the potential involvement of rhamnose in inhibiting cellular interactions.

Lewis lung carcinoma cell line, 3LL was investigated due to their ability to bind sugar residues (Kieda and Monsigny, 1986). The 3LL carcinoma cells were able to specifically recognize and rapidly bind to cells with glucose-rich receptors, while glucose-poor receptors 3LL cell line did not adhere as sufficiently when cultured with mouse pulmonary cells (Kieda and Monsigny, 1986). One aspect of the studies specifically researched adhesion inhibition, and showed glycoproteins rich in L (-) rhamnose to be a sufficient binder and a potent cell adhesion inhibitor (Kieda and Monsigny, 1986).

D (+) Xylose and L (-) Xylose

D (+) xylose 24 hours post-fertilization treatments were not effective in inhibiting the adhesion between the archenteron tip and blastocoel roof of the sea urchin gastrulas. D or L xylose did not cause statistically significant morphological outcomes that differed from the control populations. Morphologies produced by D (+) xylose treatments though not significantly different from the controls, included few non-viable embryos and exogastrulation. D (+) xylose at 30 hours post-fertilization did not produce any statistically significant effect with regard to being different from the control population either.

The L (-) xylose treatment was effective at concentration 0.0015M at 30 hours post-fertilization treatment. The 30 hour post-fertilization treatment produced statistically more unattached archenterons when compared to the control population. But this was only at one concentration at one time point.

Disaccharides: Melibiose and Trehalose

Disaccharides are thought to play a role in cellular adhesion (Fuster et al., 2003). Studies have described mechanisms in which disaccharides can either facilitate cellular adhesion or inhibit the cellular interactions. Previous studies have suggested that disaccharides and oligosaccharides play a role particularly in cell binding interactions involving sialyl Lewis X (sLe_x) (Fuster et al., 2003), is an oligosaccharide that has been identified on tumor cells that is known to bind to selectins on endothelial cells, platelets, and leukocytes that ultimately aid in tumor cell invasion (Fuster et al., 2003). Studies have shown disaccharides can potentially operate as substrate in the synthesis of additional oligosaccharides (Fuster, et al., 2003). Moreover, it is suggested that disaccharides can interfere with sLe_x on tumor cell membranes to overall disrupt metastasis (Fuster, et al., 2003). Here to test the disaccharides ability to influence sea urchin cellular interactions, we tested two disaccharides: melibiose and trehalose.

Statistically, neither carbohydrate had an effect on the sea urchin embryos. Melibiose produced few amounts of morphologies: unattached archenterons, exogastrulation, non-viable and some embryos with no invagination. In most of the treatment cohorts, melibiose was not effective in inhibiting archenteron attachment.

The majority of the morphological outcomes produced by melibiose were the complete archenteron attached; however, there were a small number of non-viable embryos as well.

Trehalose did not have an effect on the embryos; this may be due to the embryonic cells' lack of affinity for trehalose. With the 24 hours post-fertilization treatments, trehalose was able to produce small numbers of non-viable embryos and a few non-invaginated embryos.

Polysaccharides: α -Cyclodextrin

α -Cyclodextrin is a polysaccharide that has been shown in previous research to have an effect on sea urchin archenteron attachment (Sajadi et al., 2007), in that it caused embryo death. In this investigation, α -cyclodextrin had an effect on the embryos at both time points: 24 and 30 hours post-fertilization. With the 24 hour post-fertilization cohort, the carbohydrate was effective at concentrations 0.03M and 0.015M. The resulting morphologies of the embryos were mostly non-viable and show that α -cyclodextrin was very effective in killing the embryos. The only morphologies observed with this treatment were mostly non-viable with a few embryos that had complete archenteron attachment. When looking at the resulting embryo structure in comparison to the control population, instead of organized cells with optical clarity, the cells were black, dissociated, disorganized and some were scattered across the entire field of view (See Figure 6B). At the concentration of 0.015M, α -cyclodextrin was effective in producing less complete archenteron attachment and more unattached archenterons and non-viable embryos, in

comparison to the control population; resulting p-values for producing non-invaginated and non-viable were 0.009 and 0.003 respectively. This treatment produced embryos that were black in color; however, when comparing them to the 0.03M treatment, the 0.015M treated embryos were more intact and not as many cells were disassociated throughout the field of view (See figure 6C). Also, with concentration 0.003M, although it produced some completely attached archenterons, some of the archenterons were not as organized and clearly defined as seen in the control population. The optical clarity of the embryos with this group was not as clear due to the disorganization of the primary and secondary mesenchymal cells. Unlike concentrations 0.03M and 0.015M, 0.0015M dose concentrations had a few embryos with clearly defined archenterons that appeared to attach to the blastocoel wall instead of the blastocoel roof; these embryos were deemed unattached archenterons. Concentration 0.0009M statistically had no effect on the embryos; the attachment was mostly identical to the control population. Some of the archenterons treated with 0.0009M attached to blastocoel wall.

At 30 hours post-fertilization, all treatments were effective in that they differed from the control population. The trends with the 30 hour treatments were similar to the 24 hour treatments in that concentrations, 0.03M and 0.015M both produced non-viable embryos that were black in color and had disassociated cells. The other three concentrations displayed embryos with optical clarity and visible archenterons for those noted as attached archenterons. They also produced embryos that had unattached archenterons, exogastrulation, and some that were non-viable. The complete attached archenterons produced with these treatments were mostly

identical to the control; however, in some instances the archenteron was not fully elongated and therefore categorized as unattached. With this carbohydrate, the time of treatment was not a factor in that both 24 and 30 hour post-fertilization treatments produced similar results

α -Cyclodextrin is an oligosaccharide that is composed of α -(1 $\rightarrow$ 4) linked α -D-glycosyl units (Bachmann et al., 2006); because of its hydrophilic large truncated outer shape and hollow hydrophobic middle portion (that acts as an active site); it has the ability to form inclusion complexes that enable hydrophobic molecules to bind (Behr and Lehn, 1976). Molecules such as proteins and lipids are able to bind to the active site of the α -cyclodextrin hollow hydrophobic region (Behr and Lehn, 1976). So, how would these characteristics influence the embryo development? It is possible that because α -cyclodextrin has the ability to bind proteins and lipids, when it enters the embryo, it may be binding to the proteins and lipids within the plasma membrane and therefore may inhibit other surrounding cells from binding, causing disassociation of the cells within the embryo, in addition to inhibiting archenteron elongation.

Because it is well known that α -cyclodextrin binds not only to the receptors for sugars but also to the lipids and proteins, the results reported here for α -cyclodextrin cannot be easily associated with specific molecular mechanisms at this time. Although this study does not directly relate to cancer biology, the similarities of cancer cells and embryonic cells have influenced this investigation. There has been pivotal research that has been able to connect embryonic cells to cancerous cells. One study that links cancer biology to developmental biology was a study conducted by

Beatrice Mintz and Karl Illmensee (Mintz and Illmensee, 1975). In this investigation, teratocarcinoma cells were injected into mouse embryos, and the embryos into a mouse surrogate mother (Mintz and Illmensee, 1975). Results showed that once the embryos were born they were hybrid mice (Mintz and Illmensee, 1975), meaning that some of the offspring cells were derived from normal embryonic tissues and some were derived from the cancerous cells (Mintz and Illmensee, 1975). However, the mice were healthy and tumor-free (Mintz and Illmensee, 1975). This study illustrates the similarities in signaling and communication between the two cell types (Mintz and Illmensee, 1975). The cancer cells normalized during normal embryonic processes in the pregnant female mouse (Mintz and Illmensee, 1975). It also suggests that cancerous cells maybe normal cells that have been signaled and/or mutated to revert back to an embryonic-like state (Mintz and Illmensee, 1975). This information allows for parallel research findings in cancer research and developmental biology research. A breakthrough in one field may entail a breakthrough in the other. This shows the importance of understanding the beginning phases of life, with respect to intracellular and intercellular activities and environment.

Carbohydrates can also regulate morphogenesis and their associations with tumor /malignancy characteristics (Hakomori, 1984; Dube, 2005). Tumor cells can become metastatic, in which they break contact with adjacent cells (on primary tumor) and travel through the bloodstream and bind to other cells to form secondary tumors. Lectins appear to bind differently to healthy non-cancer cells compared to malignant cells (Dube, 2005). Some studies have identified specific cancer-associated cell-surface carbohydrates using antibodies and mass spectrometry (Dube, 2005). These techniques have enabled researchers to observe

conformational and molecular changes in the transformation of a malignant cell (Dube, 2005).

A study was done in 1975 in the Oppenheimer laboratory, at California State University, Northridge, to investigate and observe carbohydrate involvement in cell adhesion (Oppenheimer, 1975). In attempts to understand the basis of intercellular adhesion mechanisms in normal cells and malignant cells, this study investigated the protein Teratoma Cell Adhesion Factor (TAF) found in mouse ascites tumor cells (Oppenheimer, 1975). TAF was believed to be involved in the cell-cell adherence of malignant tumor cells (Oppenheimer, 1975). The study showed carbohydrates inhibited the binding activity of the TAF (Oppenheimer, 1975) (See Figure 3). In fact, the enzyme, β -galactosidase, inhibited TAF activity (Oppenheimer, 1975). The article further suggests that the mechanism of TAF binding may be due to TAF containing terminal D-galactosyl residues (involved in functional activity of TAF) (Oppenheimer, 1975). D-galactose was effective perhaps because it bound to the TAF cell receptors (Oppenheimer, 1975). The β -galactosidase substrate O-nitrophenyl-beta-D-galactopyranoside blocked the enzymatic activity of the β -galactosidase (Oppenheimer, 1975). The β -galactosidase acted on the TAF directly, and did not have an effect on the cell surface (Oppenheimer, 1975). Overall, this study's results suggest that the TAF contains D-galactosyl residues that are involved with the TAF adherence activity.

V. Conclusion

The most important result of this study was that some carbohydrates particularly L (-) rhamnose, and not others, statistically inhibited archenteron development and attachment. This begins to provide a clue as to what types of specific carbohydrate-binding receptors may be involved in sea urchin gastrulation events. L (-) rhamnose has been implicated in various adhesive interactions (Gabius, et al., 1994; Segura and Gottschalk, 2002). We believe that this is the first demonstration of a role for this sugar in sea urchin gastrulation in NIH designated model for studying mechanisms involved in human health and disease (Cameron, et al, 2000).

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VII. Appendix

Figure 1 Mechanism of Gastrulation

Figure 2 Mechanism for Exogastrulation

Figure 3 Carbohydrate Mediated/Cellular Interactions

This figure gives a detailed look at the carbohydrate mediated and inhibition of cellular interactions. A. demonstrates cellular interactions by showing the mechanism carbohydrates utilize to interfere and/or inhibit cell-cell adhesion. B. shows cellular interaction mechanism for carbohydrate mediated adhesion. C. is representing a lectin. D. is representing a carbohydrate chain.

Figure 4 This figure provides an overview of the morphological outcomes and population sizes of *Lytechinus pictus* embryos for each of the concentration treatments of α -Cyclodextrin at 24 Hours post-fertilization. Concentrations 0.03M and 0.015 are statistically different from the control cohort. Percentages of the morphologies are given for those that produced 7% of the population or greater. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

α -Cyclodextrin 24 hours: Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	437	0	0	0	0	437
0.03M	58	0	0	769	0	827
0.015M	329	108	71	357	29	894
0.003M	953	22	109	8	3	1095
0.0015M	898	10	63	12	2	985
0.0009M	1073	4	24	4	0	1105

TOTAL Embryo Count 5343

Table 2 Population sizes for each of the morphological outcomes. Total number of embryos used for α -Cyclodextrin 24 hours after fertilization Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 5 This figure illustrates the outcome of the different morphologies for embryos treated with α -Cyclodextrin 24 hours after fertilization. This graph gives a spread of the different morphological outcomes, including the standard error. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values α -Cyclodextrin 24 hours

Alpha 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0	1	1	0	1
0.015M	0	0.831	0.009	0.003	0.242
0.003M	1	0.396	0.999	1	0.997
0.0015M	1	0.901	1	1	0.814
0.0009M	0.493	0.998	1	1	1

Table 3 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each morphology population was compared to the control embryos at each concentration. The results specify that the α -Cyclodextrin treatment at 24 Hours post-fertilization had a significant effect, which is indicative with a p-value<0.05.

Figure 6A

Figure 6A Controls α -Cyclodextrin 24 hours
Lytechinus pictus embryos that were treated with low calcium sea water and fixed at 48 hours after fertilization. These embryos served as the control embryos for the embryos treated with α -Cyclodextrin after 24 hours post-fertilization. All of the embryos in this field of view exhibit complete archenteron (CA) attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 6B

Figure 6B. 0.03M α -Cyclodextrin 24 hours
Lytechinus pictus embryos treated with 0.03M α -Cyclodextrin at 24 hours post-fertilization. The arrows indicate the location of each embryo. These embryos are non-viable (NV). The arrows indicate the location of embryos. Magnification of this photograph is at 100x.

Figure 6C

Figure 6C. 0.015M α -Cyclodextrin 24 hours
Lytechinus pictus embryos treated with 0.015M α -Cyclodextrin at 24 hours post-fertilization. All of these embryos were categorized as non-viable. The arrows indicate the location of the embryos. Magnification of this photograph is at 100x.

Figure 6D

Figure 6D. 0.003M α -Cyclodextrin 24 hours
Lytechinus pictus embryos treated with 0.003M α -Cyclodextrin at 24 hours post-fertilization. This treatment produced unattached archenteron attachment. The arrows indicate the location of primitive archenteron cells. Magnification of this photograph is at 100x.

Figure 6E

Figure 6E. 0.0015M α -Cyclodextrin 24 hours
Lytechinus pictus embryos treated with 0.0015M α -Cyclodextrin at 24 hours post-fertilization. The embryos in this field of view were classified as having unattached archenterons. However, there were also, embryos under these conditions that had complete archenteron attachment, and some that were non-viable. The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 6F

Figure 6F 0.0009 M α -Cyclodextrin 24 hours
Lytechinus pictus embryos treated with 0.0009 M α -Cyclodextrin at 24 hours post-fertilization. Under these conditions the archenteron was more visible. The morphologies in this microscopic field of view are unattached archenterons and complete attached archenterons. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 7 This figure gives an overview of the percentages of the different morphologic outcomes and population sizes for the *Lytechinus pictus* embryos that were treated with α -Cyclodextrin at 30 Hours post-fertilization. The treatments 0.015M and 0.03M was statistically different from the control population. Percentages on the graphs represent those morphology populations of 8% and greater. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

α -Cyclodextrin 30 hour: Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	307	0	0	0	0	307
0.03M	2	0	0	1025	39	1066
0.015M	16	0	6	373	0	395
0.003M	388	35	5	5	0	433
0.0015M	315	2	1	7	0	325
0.0009M	270	18	1	2	0	291

TOTAL Embryo Count 2817

Table 4 Population sizes for each of the morphological outcomes. Total number of embryos used for α -Cyclodextrin 30 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 8 This figure illustrates the percentages of the different morphologies of the embryos treated with the sugar α -Cyclodextrin 30 hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values α -Cyclodextrin 30 hours

Alpha 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0	1	0.859	0	1
0.015M	0	0.678	1	0	1
0.003M	1	0.821	1	1	0.086
0.0015M	1	1	1	1	1
0.0009M	1	1	1	1	0.75

Table 5 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each morphology population was compared to the control embryos at each concentration. The results specify that the α -Cyclodextrin treatment at 30 Hours post-fertilization had a significant effect, which is indicative with p-values <0.05.

Figure 9A

Figure 9A Controls α -Cyclodextrin 30 hours

Control *Lytechinus pictus* embryos fixed at 48 hours after fertilization. These embryos served as the control embryos for α -Cyclodextrin at 30 hours after fertilization. They were treated with low calcium artificial seawater solely. They all demonstrate complete archenteron attachment. Embryos that were not distinguishable, like those indicated with a red arrow were not counted. Magnification of this photograph is at 100x.

Figure 9B

Figure 9B. 0.03M α -Cyclodextrin 30 hours

Lytechinus pictus embryos treated with 0.03M α -Cyclodextrin at 30 hours post-fertilization. The embryos in this field of view were noted as being disorganized and the cells within the embryos disassociate. These embryos were, therefore, categorized as non-viable. The arrows indicate the location of the embryos. Magnification of this photograph is at 100x.

Figure 9C

Figure 9C. 0.015M α -Cyclodextrin 30 hours

Lytechinus pictus embryos treated with 0.015M α -Cyclodextrin at 30 hours post-fertilization. The embryos in this field of view are non-viable. The arrows indicate the location of the embryos. Magnification of this photograph is at 100x.

Figure 9D

Figure 9D. 0.003M α-Cyclodextrin 30 hours

Lytechinus pictus embryos treated with 0.003M α-Cyclodextrin at 30 hours post-fertilization. Three morphologies are noted in this field of view, complete attachment (CA), unattached (UA) and non-viable (NV). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 9E

Figure 9E. 0.0015M α-Cyclodextrin 30 hours

Lytechinus pictus embryos treated with 0.0015M α-Cyclodextrin at 30 hours post-fertilization. Three morphologies are present in this field of view, complete attached archenterons (CA) unattached archenteron (UA) and non-viable (NV). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 9F

Figure 9F 0.0009 M α-Cyclodextrin 30 hours

Lytechinus pictus embryos treated with 0.0009 M α-Cyclodextrin at 30 hours post-fertilization. The embryos in the field of view exhibit complete archenteron attachment (CA) and unattached archenterons (UA). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 10 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of *Lytechinus pictus* embryos for each of the concentration treatments for Melibiose at 24 hours post-fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Melibiose 24 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	298	0	1	1	0	300
0.03M	935	0	0	4	1	940
0.015M	973	3	1	18	0	995
0.003M	636	0	0	2	3	641
0.0015M	330	3	2	0	1	336
0.0009M	564	2	4	2	0	572

TOTAL Embryo Count 3784

Table 6 Population sizes for each of the morphological outcomes. Total number of embryos used for Melibiose 24 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 11 This figure illustrates the percentages of the different morphologies of the embryos treated with the sugar Melibiose 24 hours after fertilization. The Melibiose treatments at 24 hours post-fertilization had no effect on the embryos in that the morphological results mirrored that of the control population. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values Melibiose 24 hours

Melibiose 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	1	0.873	1	1	1
0.015M	0.982	0.974	1	1	0.98
0.003M	0.998	0.898	1	1	1
0.0015M	0.6	1	1	0.66	0.801
0.0009M	0.612	0.663	1	1	0.962

Table 7 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each morphology population was compared to the control embryos at each concentration. The results specify that the Melibiose treatment at 24 Hours post-fertilization did not have a significant effect.

Figure 12A

Figure 12A Control Melibiose 24 hours

Control population for embryos treated with Melibiose at 24 hours post-fertilization. They are an example of complete archenteron attachment. These embryos were treated with low calcium artificial sea water. Magnification of this photograph is at 100x.

Figure 12B

Figure 12B 0.03M Melibiose 24 hours

Lytechinus pictus embryos treated with 0.03M Melibiose at 24 hours post-fertilization. All of the embryos in this image demonstrate complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 12C

Figure 12C 0.015M Melibiose 24 hours

Embryos treated with 0.015M of Melibiose at 24 hours post-fertilization. All, except one, show complete archenteron attachment to the blastocoel roof. The other shows an embryo with an unattached archenteron. The arrows indicate the location of archenteron attachment, or in the UA embryo, the arrow indicates the location of the archenteron. Magnification of this photograph is at 100x.

Figure 12D

Figure 12D 0.003M Melibiose 24 hours

These embryos demonstrate complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 12E

Figure 12E 0.0015M Melibiose 24 hours

Two *L. pictus* embryos treated with the concentration of 0.0015M Melibiose at 24 hours post-fertilization. Both show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x

Figure 12F

Figure 12F 0.0009M Melibiose 24 hours

Embryo that was treated with 0.0009M Melibiose at 24 hours post-fertilization. The embryo shows complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 13 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for Melibiose at 30 Hours post-fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Melibiose 30 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVAG	TOTAL
Control	285	0	0	0	0	285
0.03M	797	0	0	18	0	815
0.015M	536	2	2	12	0	552
0.003M	728	0	3	35	0	766
0.0015M	795	3	3	8	0	809
0.0009M	826	1	10	7	0	844

TOTAL Embryo Count 4071

Table 8 Population sizes for each of the morphological outcomes. Total number of embryos used for Melibiose 30 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 14 An illustration of the morphological results for the *Lytechinus pictus* embryos that were treated with Melibiose at 30 hours after fertilization. Statistically, Melibiose did not have an effect on the embryo morphology as most of the embryos exhibited complete archenteron attachment. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values Melibiose 30 hours

Melibiose 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	1	0.873	-	1	1
0.015M	0.982	0.974	-	0.66	0.981
0.003M	0.998	0.898	-	1	1
0.0015M	0.6	1	-	1	0.801
0.0009M	0.612	0.663	-	1	0.962

Table 9 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each morphology population was compared to the control embryos at each concentration. The results specify that the Melibiose treatment at 30 Hours post-fertilization had no significant effect, which is indicative with a p-value >0.05. The dashes indicate that there was no variance between the treatment group and the control group.

Figure 15A

Figure 15A Controls Melibiose 30 hours
Lytechinus pictus embryos treated with low calcium artificial sea water. They served as control embryos for the subset of embryos that were treated with different concentrations of Melibiose at 30 hours post-fertilization. The embryos demonstrate the morphology of complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 15B

Figure 15B 0.03M Melibiose 30 hours
 0.03M concentration of Melibiose at 30 hours post-fertilization. These embryos show complete archenteron attachment. The location of the archenteron attachments are identified via the arrows. Magnification of this photograph is at 100x.

Figure 15C

Figure 15C
 0.015M Melibiose at 30 hours. All the embryos have the same morphological outcome as the controls. The location of the archenteron attachments are identified via the arrows. Magnification of this photograph is at 100x.

Figure 15D

Figure 15D 0.003M Melibiose
30 hours

Each attachment mirrors the attachment that was displayed in the control embryos. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 15E

Figure 15E

0.0015M Melibiose 30 hours The embryos in this field view are complete attachment or non-viable. The black arrows indicate the location of attachment with those that demonstrated complete archenteron attachment (CA). The red arrow points out an embryo that was classified as non-viable (NV). Magnification of this photograph is at 100x.

Figure 15F

Figure 15F

0.0009M of Melibiose 30 hours The embryos are classified as complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 16 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for L(-) rhamnose at 24 hours post-fertilization. This sugar treatment produced had an effect shown by the increased production of each of the morphologies. The concentration 0.015M outcome was the most different from the control cohort. Percentages on the graphs represent those morphology populations at 1% and greater. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

L (-) Rhamnose 24 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVAG	TOTAL
Control	165	0	6	1	0	172
0.03M	396	212	5	19	20	652
0.015M	161	309	4	8	9	491
0.003M	231	92	72	12	2	409
0.0015M	170	47	120	6	2	345
0.0009M	145	21	74	3	1	244

TOTAL Embryo Count 2313

Table 10 Population sizes for each of the morphological outcomes. Total number of embryos used for L(-) rhamnose 24 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 17 An illustration of the morphological results for the *Lytechinus pictus* embryos that were treated with Rhamnose at 24 hours after fertilization. Statistically, the Rhamnose treatment at 24 hours post-fertilization had an effect on the embryo morphology with the concentrations indicated by as an increased production of unattached archenterons and exogastrulation occurrence with some of the concentrations. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG)

p-Values L (-) Rhamnose 24 hours

Rhamnose 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.007	1	0.302	0.751	0.001
0.015M	0	1	0.938	0.993	0
0.003M	1	0.085	1	0.956	0.524
0.0015M	0	0	1	0.998	0.953
0.0009M	1	0.084	1	1	0.999

Table 11 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. The control was compared to each morphology populations at different concentrations. The results specify that the Rhamnose treatment at 24 Hours post-fertilization had significant effect with each of the concentrations, except 0.003M and 0.009M. The treatment groups differed from the controls in that the treatments produced more unattached archenterons and more exogastrulation occurred.

Figure 18A

Figure 18A Controls of L (-) Rhamnose at 24 hours
Controls for the different concentration conditions of L(-) Rhamnose at 24 hours post-fertilization. All of which were classified as complete archenteron attachment. Magnification of this photograph is at 100x.

Figure 18B

Figure 18B 0.03M L (-) Rhamnose 24 hours
At 24 hours post-fertilization, this embryo population was treated with 0.03M of L (-) Rhamnose. The embryos were classified as either complete archenteron attachment (CA) and unattached archenteron (UA). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 18C

Figure 18C 0.015M L (-) Rhamnose at 24 hours
The embryos demonstrate two different classifications of morphologies: complete archenteron (CA) and exogastrulation (EXO). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 18D

Figure 18D

0.003M of L (-) Rhamnose at 24 hours

There are three embryos with complete archenteron attachment (CA), unattached archenteron and one with exogastrulation (EXO). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 18E

Figure 18E

0.0015M of L (-) Rhamnose 24 hours

There are three different morphologies are present in this photo: complete archenteron attachment (CA), unattached (UA), exogastrulation (EXO). The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 18F

Figure 18F

0.0009M of L (-) Rhamnose 24 hours

They were classified as unattached archenteron (UA) or exogastrulation (EXO). The arrows indicate the location of archenteron or of primitive archenteron cells. Magnification of this photograph is at 100x.

Figure 19 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for Rhamnose at 30 hours post-fertilization. This sugar treatment produced an effect indicative by the increased production of each of the morphologies. There was a significant increase in production of embryos undergoing exogastrulation. This treatment had a statistical significance with the concentrations, with an exceptions of concentrations 0.03M and 0.015M. Percentages on the graphs represent those morphology populations at 17% and greater. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG)

L (-) Rhamnose 30 hours Morphologies

	Number of Embryos					TOTAL
	CA	UA	EXO	NV	NINVAG	
Control	270	0	0	0	0	270
0.03M	893	26	43	8	0	970
0.015M	655	11	133	6	0	805
0.003M	301	14	112	4	1	432
0.0015M	382	12	200	3	6	603
0.0009M	474	15	245	2	4	740

TOTAL Embryo Count 3820

Table 12 Population sizes for each of the morphological outcomes. Total number of embryos used for L(-) rhamnose 30 hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 20 This figure represents the results from the *Lytechinus pictus* embryos treated with L(-) rhamnose at 30 hours after fertilization. This sugar caused statistically significant increase in exogastrulated and unattached embryos. These effects were observed for every concentration above the controls. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values L (-) Rhamnose 30 hours

Rhamnose 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.942	0.823	1	0.891	1
0.015M	0.522	0.201	1	0.966	0.682
0.003M	0	0.017	0.999	0.995	0.419
0.0015M	0	0	0.337	0.999	0.595
0.0009M	0.003	.001	0.763	1	0.338

Table 13 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. The each control was compared to each morphology populations at different concentrations. These results specify that the Rhamnose treatment at 30 Hours post-fertilization had significant effect with each of the concentrations with an exception of concentrations 0.03M and 0.015M. The treatment groups differed from the controls in that the treatments statistically produced more unattached archenterons, more exogastrulation occurred, and there was a decrease in the number of complete archenteron attachment.

Figure 21A

Figure 21A Controls L (-) Rhamnose 30 hours
Control embryos for those treated with L (-) Rhamnose at 30 hours post-fertilization. All of the control embryos have complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 21B

Figure 21B 0.03M of L (-) Rhamnose at 30 hours
All have complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 21C

Figure 21C 0.0015M of L (-) Rhamnose 30 hours
The different morphologies shown in this photograph are unattached, archenteron (UA) and exogastrulation (EXO). The arrows indicate where the archenteron is located, or in the case of the unattached, the location of the archenteron. The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 21D

Figure 21D 0.003M L (-)
Rhamnose 30 hours

Most of the embryos in this photograph were categorized exhibiting exogastrulation (EXO). There is one arrow indicating the location of an embryo with an unattached archenteron (UA). The embryos were treated with 0.003M of L (-) Rhamnose at 30 hours post-fertilization. The arrows indicate the location of archenteron. Magnification of this photograph is at 100x.

Figure 21E

Figure 21E 0.0015M L (-)
Rhamnose 30 hours

The embryos in this photograph mostly demonstrate exogastrulation (EXO), with the exception of one embryo that shows unattached archenteron (UA). This embryos in this photo were treated with 0.0015M of L(-) Rhamnose at 30 hours post-fertilization. The arrows indicate the location of archenteron .Magnification of this photograph is at 100x.

Figure 21F

Figure 21F 0.0009M L (-)
Rhamnose 30 hours

The embryos in this photo a show an embryo with a complete archenteron attachment (CA) and an embryo with an unattached archenteron (UA). They were treated with 0.0009M of L (-) Rhamnose at 30 hours post-fertilization. The arrows indicate the location of the archenteron or archenteron attachment. Magnification of this photograph is at 100x.

Figure 22 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for Trehalose at 24 hours post-fertilization. This sugar did not have an effect on the embryos. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Trehalose 24 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	146	0	0	0	0	146
0.03M	393	0	0	2	0	395
0.015M	264	0	0	20	0	284
0.003M	347	2	0	16	3	368
0.0015M	297	0	0	2	3	302
0.0009M	313	0	0	2	0	315

TOTAL Embryo Count 1810

Table 14 Population sizes for each of the morphological outcomes. Total number of embryos used for Trehalose 24 hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 23 This figure illustrates the results of *Lytechinus pictus* embryos treated at 24 hours after fertilization with Trehalose. This sugar produced no significant effects on morphology at any of the concentration treatments. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values Trehalose 24 hours

Trehalose 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.949	-	1	1	1
0.015M	1	-	1	0.412	1
0.003M	0.465	-	0.41	0.659	0.562
0.0015M	0.058	-	0.791	1	1
0.0009M	0.131	-	1	1	1

Table 15 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. The control was compared to each morphology populations at different concentrations. These results specify that the Trehalose treatment at 24 Hours post-fertilization did not have a statistically significant effect on the embryos. The “-” in the table indicates that there was no variance in resulting embryo morphology between the treated cohort and the control cohort in regards to the exogastrulation outcome.

Figure 24A

Figure 24A Control Trehalose 24 hours

Control embryos for the population of embryos treated with Trehalose at 24 hours post-fertilization; they were treated with low calcium sea water only. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 24B

Figure 24B 0.03M Trehalose at 24 hours

They all show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 24C

Figure 24C 0.015M of Trehalose at 24 hours
They all show complete archenteron. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 24D

Figure 24D 0.003M
Trehalose 24 hours
The arrows indicate the
location of archenteron
attachment. Magnification of
this photograph is at 100x.

Figure 24E

Figure 24E 0.0015M
Trehalose
The arrows indicate the
location of archenteron
attachment. Magnification of
this photograph is at
100x.

Figure 24F

Figure 24F 0.0009M
Trehalose 24 hours
The arrows indicate the
location of archenteron
attachment. Magnification
of this photograph is at
100x.

Figure 25 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for Trehalose at 30 Hours post-fertilization. This sugar treatment produced an effect indicative by the decreased production of complete archenteron attachment at every concentration treatment; however, there was not enough production of the other morphologies to conclude that it will cause a specific morphology. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Trehalose 30 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	230	0	0	0	0	230
0.03M	419	0	0	0	3	422
0.015M	392	0	0	3	0	395
0.003M	320	5	0	1	1	327
0.0015M	261	1	0	4	0	266
0.0009M	302	6	0	0	0	308

TOTAL Embryo Count 1948

Table 16 Population sizes for each of the morphological outcomes. Total number of embryos used for Trehalose 30 hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 26 This figure illustrates the results for the *Lytechinus pictus* embryos treated with Trehalose at 30 hours. This sugar had a statistical difference with the decrease of the complete archenteron attachment morphology outcome. The decrease, however, was very minimal. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values Trehalose 30 hours

Trehalose 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.812	-	0.884	1	1
0.015M	0.532	-	1	0.964	1
0.003M	0.347	-	0.999	1	0.878
0.0015M	0.872	-	1	0.884	1
0.0009M	1	-	1	1	0.768

Table 17 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. These results specify that the Trehalose treatment at 30 hours post-fertilization did not have a statistically significant effect. The dashes indicate that there was no variance when the morphology was compared to the control group.

Figure 27A

Figure 27A Control Trehalose 30 hours

Control population for the embryos treated with Trehalose at 30 hours post-fertilization. All the embryos in this figure show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 27B

Figure 27B 0.03M

Trehalose at 30 hours
They all exhibit complete archenteron attachment and are identical to the control group. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 27C

Figure 27C 0.015M Trehalose 30 hours

The embryos in this figure all show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 27D

Figure 27D 0.003M of Trehalose
30 hours

The embryos in this photograph were treated with 0.003M of Trehalose at 30 hours post-fertilization. There are two different morphologies present under these conditions: complete archenteron attachment and non-viable. The arrows indicate the location of the attachment, while one of the arrow points out a non-viable embryo. The non-viable embryos are very dark in color, and there is no indication of an archenteron present.

Magnification of this photograph is at 100x.

Figure 27E

Figure 27E 0.0015M Trehalose
30 hours

They all show complete archenteron attachment. The arrows indicate the location of archenteron attachment.

Magnification of this photograph is at 100x.

Figure 27F

Figure 27F 0.0009M Trehalose
30 hours

All show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 28 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for D(+) Xylose at 24 hours post-fertilization Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

D (+) Xylose 24 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVG	TOTAL
Control	210	0	0	0	0	210
0.03M	712	14	1	1	0	728
0.015M	500	5	0	0	0	505
0.003M	774	20	4	4	1	803
0.0015M	758	3	1	8	1	771
0.0009M	571	1	1	0	0	573

TOTAL Embryo Count 3590

Table 18 Population sizes for each of the morphological outcomes. Total number of embryos used for D(+) Xylose 24 Hours after fertilization Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVG).

Figure 29 This figure illustrates the different Morphologies that were present with embryos treated with D(+) Xylose at 24 hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG)

p-Values D (+) Xylose 24 hours

D(+)-Xylose 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.972	0.998	1	1	0.117
0.015M	0.826	1	1	1	0.946
0.003M	0.755	0.453	0.979	0.998	1
0.0015M	0.835	1	0.979	0.953	0.994
0.0009M	0.999	0.998	0.999	0.997	1

Table 19 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. The control was compared to each morphology populations at different concentrations. The sugar treatment did not cause any statistical differences from the controls.

Figure 30A

Figure 30A Control D(+) Xylose 24 hours

Control *Lytechinus pictus* embryos fixed at 48 hours after fertilization. The embryos served as the control embryos for the embryos treated with D(+) Xylose after 24 hours. They all exhibit complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 30B

Figure 30B 0.03M D (+) Xylose 24 hours

Some exhibit complete archenteron attachment (CA) and others show unattached archenterons (UA). The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 30C

Figure 30C 0.015M D (+) Xylose 24 hours

Some of embryos exhibit complete archenteron attachment (CA) and other unattached archenteron (UA). The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 30D

Figure 30D 0.003M D (+) Xylose at 24 hours

Most of these embryos exhibit complete archenteron attachment (CA); however, the other population present in this field of view are non-viable (NV). Magnification of this photograph is at 100x.

Figure 30E

Figure 30E 0.0015M D(+) Xylose 24

Some exhibit complete archenteron attachment (CA) and others unattached archenterons (UA). The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 30F

Figure 30F 0.0009M D(+) Xylose 24 hours

All embryos have complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 31 Overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for D(+) Xylose at 30 hours post-fertilization. This sugar treatment produced no significant effect on the embryos. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

D (+) 30 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVAG	TOTAL
Control	215	1	0	0	0	216
0.03M	608	1	1	1	0	611
0.015M	671	1	4	1	0	677
0.003M	915	0	10	1	0	926
0.0015M	755	5	10	2	0	772
0.0009M	787	0	1	5	0	793

TOTAL Embryo Count 3995

Table 20 Population sizes for each of the morphological outcomes. Total number of embryos used for D(+) Xylose 30 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 32 This graph illustrates the different morphologies that resulted from *Lytechinus pictus* embryos treated with D(+) Xylose 30 hours after fertilization. This treatment had no effect on the embryos. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values D (+) Xylose 30 hours

D(+)-Xylose 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	1	1	-	1	0.976
0.015M	0.997	0.96	-	0.999	0.98
0.003M	0.342	0.342	-	1	0.873
0.0015M	0.965	0.342	-	0.989	0.976
0.0009M	0.9	1	-	0.624	0.873

Table 21 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. The each control was compared to each morphology populations at different concentrations. These results specify that the D(+) Xylose treatment at 24 Hours post-fertilization had no significant effect with any of concentration treatments.

Figure 33A

Figure 33A Control D (+) Xylose 30 hours

Control *Lytechinus pictus* embryos fixed at 48 hours after fertilization. These embryos served as the control embryos for the embryos treated with D(+) Xylose after 30 hours. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 33B

Figure 33B 0.03M D (+) Xylose 30 hours

All the embryos in this field of view exhibit complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 33C

Figure 33C 0.015M D (+) Xylose at 30 hours

All of the embryos in this field of view demonstrate complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 33D

Figure 33D 0.003M D (+) Xylose 30 hours

These embryos have attached archenterons (CA) and unattached archenterons (UA). The unattached embryos in this field view are disorganized. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 33E

Figure 33E 0.0015M D (+) Xylose at 30 hours

These embryos have attached archenterons (CA), there is one embryo that is non-viable (NV), indicated with the red arrow. The black arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 33F

Figure 33F 0.0009M D (+) Xylose at 30 hours

These embryos have attached archenterons. The arrows indicate the location of archenteron attachment. Magnification of this photograph is at 100x.

Figure 34 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for L(-) Xylose at 24 hours post-fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

L (-) Xylose 24 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVAG	TOTAL
Control	444	0	0	0	0	444
0.03M	1029	4	29	6	0	1068
0.015M	798	2	7	20	0	827
0.003M	761	2	5	5	0	773
0.0015M	645	9	28	4	1	687
0.0009M	542	1	13	3	0	559

TOTAL Embryo Count 4358

Table 22 Population sizes for each of the morphological outcomes. Total number of embryos used for L(-) Xylose 24 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 35 This figure represents the result morphologies of the embryos at 24 hour incubation with L(-) Xylose,. Under these conditions, the L(-) Xylose had no effect on the embryo morphology at any of the concentrations. Marjority of the embryos had complete archenteron attachment. . Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values L (-) Xylose 24 hours

L(-)Xylose 24 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.218	0.059	1	0.979	0.949
0.015M	1	0.998	1	0.176	0.998
0.003M	1	1	1	0.991	0.998
0.0015M	1	0.41	0.85	0.997	0.341
0.0009M	1	0.963	1	0.999	1

Table 23 This table illustrates p-values for L (-) Xylose 24 hours post-fertilization that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each control was compared to each morphology populations at different concentrations. There was no significant effect with any of the concentrations.

Figure 36A

Figure 36A Controls L (-) Xylose
24 hours

Control *Lytechinus pictus* embryos used for embryos treated with L (-) Xylose at 24 hours after fertilization. The embryos in figure A were solely treated with low calcium sea water. They exemplify the morphology of complete archenteron attachment. Magnification of the field of view is 100x.

Figure 36B

Figure 36B 0.03M L (-) Xylose 24
hours

All four of the embryos in the field view show complete archenteron attachment. Magnification of the field of view is 100x.

Figure 36C

Figure 36C 0.015M L (-)
Xylose at 24 hours

All the embryos in this field of view have complete attached archenterons. Magnification of the field of view is 100x.

Figure 36D

Figure 36D 0.003M L (-) Xylose
24 hours

This figure illustrates L (-) Xylose treatment at 24 hours after fertilization. The embryos were treated with L (-) Xylose with a concentration of 0.003M. All have complete attached archenterons. The arrows indicate the location of archenteron attachment. Magnification of the field of view is 100x.

Figure 36E

Figure 36E 0.0015M L (-) Xylose
24 hours

The embryos in this figure were treated with 0.0015M of L (-)Xylose at 24 hours post-fertilization. All demonstrate the complete archenteron attachment morphology. The arrows indicate the location of archenteron attachment. Magnification of the field of view is 100x.

Figure 36F

Figure 36F 0.0009M L (-) Xylose
24 hours after

All show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of the field of view is 100x.

Figure 37 This figure provides an overview of the percentages of the different morphologic outcomes and population sizes of the *Lytechinus pictus* embryos for each of the concentration treatments for L(-) Xylose at 30 hours post-fertilization. This sugar treatment statistically produced a slight increase of unattached embryos and a slight decrease in complete archenteron attachment occurrence at concentration 0.0015M. Percentages on the graphs represent those morphology populations 12% and greater. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

L (-) Xylose 30 hours Morphologies

Number of Embryos						
	CA	UA	EXO	NV	NINVAG	TOTAL
Control	148	0	0	0	0	148
0.03M	293	41	0	0	12	346
0.015M	208	31	2	0	0	241
0.003M	242	42	0	0	0	284
0.0015M	200	51	0	0	1	252
0.0009M	242	7	0	0	0	249

TOTAL Embryo Count 1520

Table 24 Population sizes for each of the morphological outcomes. Total number of embryos used for L(-) Xylose 30 Hours after fertilization. Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

Figure 38 This figure represents results for *Lytechinus pictus* embryos with 30 hour incubation with L(-) Xylose. Concentrations 0.015M produced statistically significant increases in the amount of unattached embryos and statistically significant decreases in the amount of completely attached embryos. . Abbreviations: Complete Archenteron (CA), Unattached Archenteron (UA), Exogastrulation (EXO), Non-viable (NV), No Invagination (NINVAG).

p-Values L (-) Xylose 30 hours

L(-)Xylose 30 p-Values	Complete Attached Archenteron	Exogastrulation	No Invagination	Non-Viable	Unattached Archenteron
0.03M	0.343	1	0.289	-	0.249
0.015M	0.401	0.867	1	-	0.568
0.003M	0.1	1	1	-	0.224
0.0015M	0.034	1	1	-	0.019
0.0009M	0.1	1	1	-	0.999

Table 25 This table illustrates p-values that resulted from a One-Way ANOVA, followed by a Tukey Pairwise Comparison. Each control was compared to each morphology populations at different concentrations. The L(-) Xylose (0.0015M) treatment at 30 Hours post-fertilization had an effect in producing slightly more unattached archenterons, and a slight decrease in complete archenteron attachment. Otherwise, there was no significant effect with any of the other concentrations. Dashed lines indicate no variance between the control group and the treatment group.

Figure 39A

Figure 39A Control L (-)Xylose 30 hours

Control embryos for those treated with L(-)Xylose after 30 hours. These embryos in particular were treated with low calcium artificial sea water at 30 hours. They show complete archenteron attachment. The arrows indicate the location of archenteron attachment. Magnification of the field of view is 100x.

Figure 39B

Figure 39B 0.03M L (-)Xylose 30 hours

They all show complete archenteron. The arrows indicate the location of archenteron attachment. Magnification of the field of view is 100x.

Figure 39C

Figure 39C 0.015M L (-) Xylose after 30 hours

The embryos with the large dot in the center were not counted due to the inability to say confidently that attachment occurred (and example is indicated with a red arrow). Also, some of these embryos were categorized as being unattached while others were categorized as attached due to their attachment to the wall of the embryo proper. The arrows indicate the location of the archenteron. Magnification of the field of view is 100x.

Figure 39D

Figure 39D 0.003M L(-) Xylose with 30 hours
The embryos in this field view exhibit the unattached (UA) morphology. The arrows indicate the location of the archenteron. Magnification of the field of view is 100x.

Figure 39E

Figure 39E 0.0015M L (-) Xylose 30
These embryos demonstrate morphologies of unattached archenterons (UA) and exogastrulation (EXO). The arrows indicate the location of the archenteron. Magnification of the field of view is 100x.

Figure 39F

Figure 39F 0.0009M L (-)Xylose at 30 hours
Notice the unattached (UA) and exogastrulated (EXO) archenterons. The arrows indicate the location of the archenteron. Magnification of the field of view is 100x.