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INHIBITION OF CELL AGGREGATION
BY SPECIFIC CARBOHYDRATES

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requirements for the degree of Master of Science in

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by

Michiko Irene Asao

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The Thesis of Michiko Irene Asao is approved:

Dr. ~~Richard~~ Potter

Dr. Donald Bianchi

Dr. ~~Steven~~ B. Oppenheimer

California State University, Northridge

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ABSTRACT

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One approach to investigating the potential role of surface carbohydrates in mediating intercellular adhesion is to study cell reaggregation in the presence of defined concentrations of specific saccharides. Fifteen different exogenously added saccharides were tested for their effect on the reaggregation of 24 hour sea urchin embryo cells (Stongylocentrotus purpuratus) dissociated by removal of divalent cations. Aliquots (0.2 ml) of cell suspension were rotated at 68 rpm, 17°C, pH 8.0 with varying concentrations (10^{-1} to 10^{-5} M) of the sugars. Relative percents of cell aggregation were determined using an electronic particle counter assay. In all experiments cell viability using trypan blue was over 95.8%. Among the

sugars tested, in 15 separate experiments, D-galactose and N-acetyl-D-galactosamine consistently inhibited aggregation to the greatest extent at all time points. D-galactose, at all concentrations tested, at 10, 20, 30, 40, and 60 minutes rotation, showed mean decreases of aggregation over control values in the absence of sugar of 59.3%, 53.6%, 43.2%, 35.0% and 36.4% respectively. N-acetyl-D-galactosamine also caused mean decreases in aggregation of 73.5%, 54.5%, 40.8%, 42.2%, and 45.6% respectively. Each difference over the control is significant to the p value of less than 0.01. In three experiments, β -galactosidase substantially inhibited reaggregation of these cells. These results suggest that galactopyranosyl-like groups may be implicated in mediating adhesion of 24 hour sea urchin embryo cells to each other.

INTRODUCTION

The mechanism of cell adhesion has been a challenging question in recent years. A solution to this problem cannot be attempted without an understanding of the nature of the plasma membrane. Since the conceptual revision of the membrane structure to the 3-D fluid mosaic model proposed by Singer and Nicolson (1972), the possible diversity of functions attributable to this organelle seems limitless. Although the evidence is not conclusive, functional processes such as cell growth, cell movement, and recognition would appear to be dependent upon the cell membrane either by direct mediation or some type of responsive translational mechanism following stimulation.

There are several theories which have been proposed to explain cell adhesion. Two of which will be discussed here because they have direct relevance to this project. The first hypothesis of membrane adhesion is the Tyler-Weiss model (Tyler, 1940; Weiss, 1941) in which there is an antigen-antibody binding process taking place between an antigen-like membrane component and a receptor on an adjacent cell surface. If two cells are compatible, that is, the membrane surfaces both contain the complimentary units,

adhesion can occur providing contact is allowed. This model can explain developmental phenomena where cells are known to travel across one another during morphogenesis (Gustafson and Wolpert, 1967; Barbera et al., 1973).

Migratory cells not having the complimentary surface antigens of adjacent cells will be unable to adhere to one another and consequently their movement will not be inhibited. Cells in which surface antigens needed for specific adhesion have been incorporated into the membrane will undergo adhesion to one another and further cell migration will be halted.

It has been suggested that the arrangement of the membrane components permits the specificity of the adhesion (Steinberg, 1963) and that the attractions are largely polar in nature. Arrangements within as well as across the membrane are possibly unique to particular cell types, and therefore this contributing factor should not be ignored. However, the formal discussion presented by Bell (1978) on reversible bonds implies that forces of adhesion found between cells are far greater than those which can be explained by simple electrostatic forces.

The functional antigens creating adhesions between cells might be proteinaceous (Moscona et al., 1963, Guidice 1965) or carbohydrate (Lemon et al., 1971; Cuatrecasas, 1973; Strobel, 1973; Turner and Burger, 1973) in nature. Receptors for ligand binding have been studied to a lesser

extent. A protein receptor has been identified, however, by Weinbaum and Burger (1973). Proteins and carbohydrates would seem to be ideal as antigenic molecules since they are known to exist in a variety of structures among living systems.

The unusual adhesive behavior of transformed cells has been explained by the change in topography of the membrane (Burger, 1969) or by de novo synthesis and rapid turnover depletion of certain surface glycoproteins (Hynes, 1976). Both theories can in fact be incorporated into the ligand-receptor model when considering the possible requirement of distribution and concentration of molecules within the membrane. Lectins, which are carbohydrate binding molecules, have been very useful in studying the surface characteristics of the plasma membrane. Changes in fluidity of the membrane (Roberson et al., 1975) and differences in distribution of certain glycoproteins (Neri et al., 1975) have been detected. Nicolson (1971) observed that the distribution and mobility of molecules binding Concanavalin A differed dramatically between transformed and normal 3T3 fibroblasts. He found that the transformed cells had a random distribution of Con A receptor sites compared to the clustering of sites on normal fibroblasts. Roberson et al. (1975) observed increased mobility of Con A receptor sites on micromeres of sea urchin embryo cells which are the most motile cell type. These results demon-

strate that the mobility and distribution of certain receptors in the plasma membrane among different types of cells correlates with different behavioral characteristics.

Perhaps the adhesion of cells requires the arrangement of antigens in a definite pattern complimented by the appropriate pattern on an adjacent cell.

The second mechanism of cell adhesion to be described was proposed by Roseman (1970). His theory states that externally exposed glycosyltransferases attach to sugars on the surface of adjacent cell membranes. Evidence has shown that these enzymes are located on plasma membranes (Roth et al., 1971; Roth and White, 1971). Roseman suggests that the reaction which requires the binding of the substrate to the enzyme would cause an adhesive bond between the two cells. This model can explain three of the observed contact phenomena. (1) The specificity of cell adhesions may be explained by the known requirements of enzyme-substrate bonds (Lehninger, 1975). (2) The contact inhibition exhibited by normal cells is precipitated by the catalytic activity from adjacent cells (Roth and White, 1972). (3) The migration of cells during morphogenesis can be directed by the activity of specific surface enzymes. Migratory cells which have incorporated particular glycosyltransferases into their membranes will be able to form enzyme-substrate bonds with adjacent cells. These adhesive bonds will determine the ultimate positioning of migratory

cells for normal development (Shur et al., 1975; Shur, 1977).

Carbohydrate rich coats are known to exist quite commonly on many cell types (Rambourg and Leblond, 1967). This does not seem unusual since glycolipids and glycoproteins are naturally occurring membrane components which are synthesized by the cell (Cook et al., 1965; Eylar, 1965; Cook and Eylar, 1965; Hakomori and Murakami, 1968; Bosmann et al., 1969). It would appear that these carbohydrate chains are the first membrane particles that an approaching cell would encounter and thus they are a likely candidate as the means by which specific recognition could occur. In both mechanisms described above, carbohydrate chains could render themselves as functional units in adhesion. The antigen-antibody hypothesis could utilize sugars on glycoproteins as antigens with the specificity incorporated by the terminal sugar structure (Kabat, 1956; Landsteiner, 1962; McKenzie et al., 1977). The glycosyltransferase hypothesis would necessarily require the enzyme to bind an oligosaccharide chain as one of its substrates.

Evidence to support the proposed involvement of heterosaccharides in cell adhesion was provided by Oppenheimer et al. (1969). They found that single cells obtained by trypsin treatment of "embryoid bodies", the ascites grown form of mouse teratoma, aggregated in a complex tissue medium but not in Hanks' balanced salts

solution. The researchers were able to isolate, out of 51 components of the medium, the one active component, L-glutamine, which enabled the aggregation to proceed. Omission of this ingredient prevented aggregation. It was also discovered that D-glucosamine and D-mannosamine promoted the process without L-glutamine. From this information, Oppenheimer et al. (1969) suggested that the teratoma cells require L-glutamine for the synthesis of amino sugars which will ultimately be incorporated into external sugar complexes involved in intercellular adhesion. This pathway of biosynthesis has also been demonstrated in other ascites tumor and embryonic cells (Oppenheimer and Humphreys, 1971; Oppenheimer, 1973).

Karp and Solursh (1974) have suggested that polysaccharides are important to the cell surface morphology and cell movement of sea urchin embryo cells. The experimenters noted that 90% of the polysaccharide synthesized during the blastula stage become sulfated and if embryos were allowed to develop in media without sulfate, a considerable decrease (70%) in those sulfated polysaccharides would result. Primary mesenchyme cells of embryos that were raised in sulfate free media were shown to lack migratory ability at the appropriate time of gastrulation. Scanning electron microscopy of the mesenchyme membrane of embryos raised in non-sulfated media was smooth as opposed to the normal rough appearance. These observations could

reflect the absence of the extracellular coat required for normal cellular interactions. In light of the contention presented by Steinberg (1963) that cell migration might be determined by differential and selective adhesiveness, the carbohydrate coat observed by Karp and Solursh might be involved in the adhesion of sea urchin mesenchyme cells. Gustafson and Wolpert (1967), in a review of sea urchin morphogenesis, also attribute the movement of mesenchymal cells during gastrulation to a variation of adhesiveness.

Spiegel and Spiegel (1975) have shown that there is a qualitative difference between species specific and non-specific adhesions of reaggregating sea urchin cells. During reaggregation, cells of the same species form microvilli and produce an extracellular electron dense material which looks similar to the hyalin layer in appearance. Cells of different species do not form microvilli or the extracellular material during reaggregation. Spiegel and Spiegel suggest the extracellular substance is composed of the molecules described by Tonegawa (1973) and Kondo (1973).

Tonegawa (1973) has isolated and identified mucopolysaccharide protein complexes which promoted the aggregation of dissociated sea urchin embryo cells. This component, regarded as particulate aggregation factor (PAF) by Tonegawa, was isolated from the calcium and magnesium free supernatant of dissociated sea urchin embryos. It was determined that the PAF was extracted from the membrane surface.

The experimenter suggests that these cell surface mucopolysaccharides are stabilized in the membrane by calcium and that together, PAF and calcium, are the essential units for adhesion to occur. The analytical results indicate that the PAF is rich in acidic groups. For a single histidine and methionine residue, 2.4 moles sialic acid and 12.6 moles sulfate are involved. It has been estimated that 20.0% of the amino acids present in the molecule are acidic. Tonegawa implicates all three types of acidic substituent groups to be involved in the aggregation of cells. The acidic particulate aggregation factors are hypothesized to combine via calcium to form bridges that bind acidic groups on adjacent cell surfaces. Tonegawa implies that the aggregation promoting molecule described by Kondo (Kondo and Sakai, 1971; Kondo, 1973) is the protein portion of the PAF mucopolysaccharide.

The molecules discovered by Tonegawa and Kondo can explain the sorting out phenomena observed by Spiegel and Spiegel (1975). Mixtures of embryonic cells of the sea urchin Arbacia punctulata, and of the sand dollar, Echinarachnius parma, initially adhered to one another, but after incubation, sorting out of cells according to species occurred and aggregates were composed of cells of either one or the other species. The researchers propose that the extracellular material allows specific binding of similar cells and that its absence, as found in non-similar cell

type aggregates, would insure uninterrupted passage over one another. These findings implicate carbohydrates to be functional in sea urchin cell adhesion when combined with the information presented by Tonegawa. Models for the mechanism by which adhesion occurs in the sea urchin could be similar to those ascribed to sponge reaggregation (Lemon et al., 1971; Turner and Burger, 1973; Weinbaum and Burger, 1973; Burger, 1975).

Glycosyltransferases have been reported to exist in the sea urchin eggs and embryos of Arbacia punctulata (Schneider and Lennarz, 1976). Schneider and Lennarz observed the behavior of two of the membrane bound transferases, mannosyl- and galactosyltransferases, for their activity during development. The experiments performed showed a constant rate of activity for the mannosyltransferases as opposed to the 10-fold increase in activity displayed by the galactosyltransferases which occurs at gastrulation. The researchers suggest that this differential behavior among transferases could implicate their involvement in the cell-cell interactions that take place during morphogenesis.

The present study was performed to determine if carbohydrates are important functional membrane components of cell-cell adhesion in gastrulating sea urchin embryos and, if carbohydrates are involved, which molecular structure is most active. The protocol involved a series of hapten

inhibition assays on the reaggregation of dissociated 24 hour sea urchin embryo cells (Strongylocentrotus purpuratus).

The sea urchin system has two advantages for this study. First, the embryos dissociate with simple washing with calcium and magnesium free sea water which minimizes any damage to the cell membrane. Second, when provided with normal sea water the cells will reassociate without addition of foreign chemicals presumably by means that approximate in vivo processes.

MATERIALS AND METHOD

Reagents and media

Sea urchins (Strongylocentrotus purpuratus) were purchased from Pacific Bio-Marine, Venice, CA.

D(-)-Arabinose	α -L-fucose
D(+)-galactose	α -D-galacturonic acid
α -D-glucuronic acid	maltose
D(+)-mannose	N-acetyl-galactosamine
N-acetyl-glucosamine	N-acetyl-mannosamine
L(-)-sorbose	stachyose
D(+)-xylose	ethylenediamine tetraacetic acid (EDTA)

and Deoxyribonuclease I (DNase) 1x crystallized and lyophilized were obtained from Sigma Chemical Co., St. Louis, Missouri. β -D(-)-Fructose, β -D-glucose, 2-amino-2(hydroxymethyl)-1,3-propandiol (Tris), and trypan blue dye were purchased from Matheson, Coleman, and Bell, Norwood, Ohio. Citrate buffer was made by a mixture of sodium citrate (Merck & Co., Inc. Rahway, N.J.) and citric acid (J.T. Baker Chemical Co., Phillipsburg, N.J.).

All glycosidases were purchased from Sigma Chemical Co., St. Louis, Missouri with the following specifications:

Enzyme	Activity units/mg prot	pH optimum	mg prot/mg	Contamination
α -L-fucosidase	2-3	6.5	0.364	1.5% β -N-acetyl-glucosaminidase
β -galactosidase	200-400	7.3	solid	0.01% α -galactosidase α -glucosidase
α -glucosidase	9	7.6	solid	0.01% β -glucosidase α & β -galactosidase
α -mannosidase	15-25	4.5	5.1	0.05% β -galactosidase β -N-acetyl-glucosaminidase α -galactosidase α -L-fucosidase
β -N-acetyl-glucosaminidase	35-50	4.0	0.25	0.5% β -galactosidase α -galactosidase β -xylosidase

Calcium-magnesium free sea water (CMF-SW) was prepared as follows: 27.0g NaCl, 1.0g Na₂SO₄, 0.8g KCl, and 0.18g NaHCO₃ were dissolved in one liter of distilled water. 0.02M Tris buffer was added to adjust pH to 8.0. Millipore filtered sea water (MF-SW) was obtained by filtering sea water through 0.45 μ and 0.22 μ millipore filters which will eliminate protozoa and debris.

Preparation of cell suspension

Gametes of the sea urchin Strongylocentrotus purpuratus were obtained by intercoelomic injection of approximately 1-2 ml of 0.55M KCl (Barber, 1971). Sperm was kept on ice while the egg suspension was washed three times in 0.02M Tris buffered millipore filtered sea water (MF-SW) at pH 8.0. 5 ml of lightly packed eggs were diluted with 50 ml of MF-SW and 0.1 ml of undiluted sperm was added. The suspension was slowly stirred for 45 seconds and then poured into two liters of MF-SW to prevent clumping of the zygotes. The two liter suspension was then distributed into petri plates and allowed to develop at 17° C for 24 hours.

After 24 hours of incubation, sea urchin embryos are hatched and swimming. Therefore, this provides a convenient way to insure collection of only fully developed embryos. The contents of the petri plates were placed in a large two liter beaker and embryos were allowed to swim

within the medium. Debris, unfertilized eggs and undeveloped zygotes settled to the bottom. The embryos within the supernatant were pelleted by low speed centrifugation (250 rpm) on a desktop International Clinical Centrifuge.

Dissociation of embryos was carried out by procedures described by Krach et al. (1974). The suspension was washed three times in 0.02M Tris buffered calcium-magnesium free sea water (CMF-SW) at pH 8.0. 0.2 ml of 0.01M EDTA in CMF-SW solution was added to each ml of packed eggs suspended in 10 ml of CMF-SW. The mixture was incubated for 10 minutes at 17° C and then gently pipetted ten times with a Pasteur pipetted to complete dissociation. The cell suspension was then centrifuged at high speed (1420 rpm) and resuspended in MF-SW with 10 µg DNase/ml. Final concentration of the cell suspension was approximately 2×10^6 cells per ml.

Experimental protocol

Saccharide solutions were made at concentrations varying from 10^{-1} to 10^{-5} M in MF-SW by serial dilutions. Within 1 dram screw cap vials, 0.2 ml of the sugar solution and 0.2 ml aliquots of the cell suspension were rotated together at a speed of 68 rpm on a gyratory shaker with a $4\frac{5}{8}$ inch diameter of rotation at 17° C. At various intervals (generally 0, 10, 20, 30, 40, 60, 90, 120, and 150 minutes) vials were removed, their contents diluted to 10

ml in MF-SW, and counted with an electronic particle counter. Routine examination for viability of cells was performed using 0.1M trypan blue dye (Krach et al., 1974; Oppenheimer et al., 1969) which will reveal dead or damaged cells by the uptake of the dark blue stain.

Glycosidase experiments were all executed within the pH range indicated as optimum for each enzyme. Following dissociation, cells were pelleted at 1420 rpm and resuspended in 0.02M citrate buffered MF-SW at pH 4.0, 4.5, and 6.5. 0.02M Tris buffered MF-SW was used to buffer at pH 7.3 and 7.6. Glycosidases (at differing concentrations which were determined by availability) were mixed with cells and allowed to incubate for 30 minutes at 17° C. Following hydrolysis, suspensions were pelleted at 1420 rpm and resuspended in MF-SW at pH 8.0. Aliquots were distributed into 1 dram vials and counted at regular intervals. To observe the contributing effects of cell incubation at differing pH's (other than pH 8.0), controls (without enzymes) were performed.

A quantitatively reliable method for measuring cell aggregation was developed and described by Oppenheimer and Odencrantz (1972). This assay measures the disappearance of single cells into aggregates in a suspension employing an electronic particle counter. In this study, a Model 112 LT Celloscope (Particle Data, Inc., Elmhurst, Ill.) was utilized to measure single cell number. The settings were;

current = $\frac{1}{2}$ and gain = 24. The window range settings for single cells were 10-90 which excluded debris. The decrease in number of single cells as a function of time was due to formation of aggregates and not cell lysis as confirmed by microscopic examination, dye exclusion tests, and electronic particle debris window readings. A few experiments were performed on a Model B Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) with similar results as found using the Model 112 LT Celloscope. The Coulter Counter settings were $\frac{1}{\text{amp}} = \frac{1}{2}$ and current = $\frac{1}{2}$. The window settings were 10-70.

Statistical analysis were performed by methods described by Sokal and Rohlf (1969). Significant values were determined using statistical tables (Rohlf and Sokal, 1969). One asterisk (*) designates a value significant to the $p < .05$ level and two asterisks (**) indicates a significant value of $p < .01$. Fisher's F or "t" values which were not significant to the $p > .75$ level were labeled NS.

RESULTS

Reliability of Data

All experiments in the present study were repeated three times. Results were highly reproducible under the standard conditions of the assay procedure. In five experiments, replicate determinations of cell number between two vials containing 0.2 ml of cell suspension and 0.2 ml of MF-SW at 60 minutes showed a mean difference value of 2.9% with a standard deviation (SD) of 1.9%. This amount of error can be attributed to inherent variables of the system, for example, from differences in the geometry of the vials, pipetting, and the electronic particle counters.

Decreases in cell number as a function of time was a result of aggregate formation of viable cells and not cell lysis. Cell viability was determined microscopically using trypan blue dye exclusion tests which exhibited a mean value of 96.2% viable cells with a standard deviation of 2.3% among the saccharide experiments. The glycosidase experiments rendered a slightly lower percentage of viable cells (95.8%) with a larger range (SD = 3.2%).

Saccharide Regression Data Analysis

Rates of adhesion can be derived from the slope of a line generated by the decreasing number of single cells as a function of time (Orr and Roseman, 1969). To generate a reliable linear relationship, a transformation of the single cell number to $\log_{10}$ is necessary. This transformation and the needed statistics for regression analysis were made available by the BMD 05R program (Table 1). Each data point from which the linear regression was determined was the mean value of three experiments. All regression lines demonstrated a high correlation coefficient significant to the $p < .05$ level with the exception of N-acetyl-glucosamine at $10^{-3}M$ (Table 1).

The regression coefficient can be derived from the regression equation of the predicted line $Y = a + bX$, where a is the y intercept. Therefore, the regression coefficient is the slope of the linear regression. Keeping in mind that a perfectly horizontal line has the slope of zero, we would expect in a case where two lines are originating from an identical point, that one slope would be less, and consequently the rate, if the minimum cell number approached is greater than that of the other. As seen in Table 1, all saccharide solutions ranging from concentrations of 10^{-1} to $10^{-5}M$ have a lower regression coefficient from its control implying a decrease in the rate of adhesion, with

the exception of N-acetyl-mannosamine at 10^{-3}M , two galacturonic acid experiments at 10^{-4}M and 10^{-5}M , xylose at 10^{-5}M , stachyose at 10^{-2}M , and all five maltose concentrations. It is not appropriate to compare two regression coefficients by t-tests utilizing standard errors. Regression analysis must be computed to test the significance between two regression lines. F values were calculated for each sample and none of the sugars at any concentration showed a significant difference from the control value at the $p < .05$ level. This fact is largely due to the logarithmic transformation. Figure 1 shows the effects of three different sugars (mannose, glucose, and galactose) at the concentration of 10^{-2}M on the rate of adhesion.

The lack of significant differences between control and sample values did not discourage the original hypothesis that saccharides, if functional in adhesion, would somehow inhibit aggregation and subsequently the rate. Upon inspection of the regression coefficients, one can observe a consistent decrease in rate which implies a rather small but detectable contribution to inhibition except in the case of maltose. Maltose, for all five concentrations of exogenously added sugars, promoted aggregation, however, once again not to a significant degree.

Throughout all five concentrations of any given sugar, no saccharide displayed any concentration dependent behavior with the possible exception of galacturonic acid

Figure 1. Regression lines of dissociated 24 hour sea urchin embryo cells reaggregated with exogenously added monosaccharides. The slope of the lines indicate the rate of adhesion taking place. Monosaccharides were added at a concentration of $10^{-2}M$ to a cell suspension and rotated at 68 rpm on a shaker. Sugars utilized in this experiment were: (1) $\square$ mannose; (2) $\circ$ glucose; (3) $\blacksquare$ galactose; and (C) $\bullet$ the control. Regression lines were generated from six points. Each point was the calculated average of three data values.

(Table 1). Two possibilities were entertained at this point; (1) the sensitivity of the assay procedure was not adequate for revealing such small changes in the rate of aggregation or (2) at $10^{-5}M$, as with all greater concentrations, maximum inhibition was taking place. The first contention is an unavoidable inevitability but does not invalidate subsequent steps which will follow if assumption 2 is accepted. The second assumption was decreed admissible from examination of the 95% confidence limit value (Table 1) for each regression coefficient. With the exception of extreme cases, all regression coefficient values overlapped one another with their respective 95% confidence intervals suggesting that the slopes could have been generated by the same points with reasonable probability. Therefore, all subsequent analysis will be analyzed with this premise in mind, specifically, each experiment at differing concentrations will be considered similar or as replicates of one another.

Mean values of the five experimental regression coefficients for a specific saccharide were calculated and their standard deviations are displayed in Table 2. Percent differences from respective control values were computed which indicate decreases in rates of adhesion for all fifteen sugars. Column 6 in Table 2 orders the sugars from the most highly reduced rate to least reduced rate (1 - 15). Galactose is noted to be the most inhibitory of adhesion

shown by a 33.0% reduction followed by 6 sugars which show a 20% reduction. Significance testing of mean sample regression coefficients with controls were obviously unnecessary since individual experimental values were determined NS by previous tests.

Saccharide Percent Aggregation Analysis

Cell number counts were taken at time intervals of 0, 10, 20, 30, 40, 60, 90, 120, and 150 minutes. Experimental control results indicated that maximum single cell loss would occur at 60 to 90 minutes from the onset of rotation and then level off so that no appreciable change could be observed in the remaining 60 minutes. Reaggregation of cells in media which contained exogenously added sugars showed reduced amounts of aggregation initially (from 0 to 60 minutes) but then would slowly approximate control values during the remaining 90 minutes. This observation would be expected in a dynamic adhesion model.

Percent aggregation was calculated by comparing the single cell count at any particular time to that of its zero time reading. Mean values of the saccharide experiments were determined from all fifteen data values since no sugar demonstrated any concentration dependent behavior. Mean values and their standard deviations are tabulated on Table 3.

In column 3, percent differences from control percent

aggregation are displayed which is a measurement of the amount aggregation was inhibited ostensibly by the added saccharide. Once again, the saccharides are ordered 1 through 15 on the basis of their inhibitory ability. Columns 5 and 6 are t-test values using a two sample t-test to determine the significance of the difference between the sample and control means.

The data for all five time points (10, 20, 30, 40, and 60 minutes) are presented on tables 3, 4, 5, 6, and 7 respectively, so that trends can be easily viewed. An interesting observation to note is the consistent rank of some of the saccharides. Specifically, N-acetyl-galactosamine and galactose appear to inhibit the reaggregation of dissociated sea urchin embryo cells to the greatest extent as early as 10 minutes after incubation. Throughout the entire experiment, at all time points, these two sugars consistently reveal themselves to be the most powerful inhibitors with N-acetyl-glucosamine and glucose following third and fourth in rank. All four of the sugars mentioned here showed their inhibitory effects to be significantly different from control means to the $p < .05$ level for all time points.

Glycosidase Regression Data Analysis

Dissociated 24 hour sea urchin embryo cells were incubated with the following amounts of enzymes; 100 μ l of

α -L-fucosidase, α -mannosidase, and β -N-acetyl-glucosaminidase, 1.0 mg of α -glucosidase, or 20 mg of β -galactosidase in 5 ml of cell suspension. Specifications of enzymes are previously described in the Materials section. The concentration of the enzymes were determined so that three experiments could be performed. Effects received indicate sufficient amounts were adequately applied. The mean time value of three data points were calculated. Single cell number was transformed to $\log_{10}$ and graphed as a function of time. The program BMD 05R computed the best fitting regression line and produced the appropriate statistics (Table 8). Significance testing for the correlation is displayed in Table 8. All lines except for that of galactosidase had a p value of less than .05. F values were calculated for regression analysis between enzyme experimental coefficient values and their respective controls. All were found not to be significant.

Displayed in Table 9 are the determined values for the percent differences between sample regression coefficients and controls. This value indicates the amount of rate reduction from control values. Enzymes were then numbered in order of effectiveness in reducing the rate of adhesion (1 through 5). Galactosidase can be observed to have the greatest effect compared to its control as shown in Figure 2.

Figure 2. Effect on reaggregation of dissociated 24 hour sea urchin embryo cells by galactosidase treatment. Cells were incubated with the enzyme for 30 minutes, washed, and rotated at 68 rpm on a gyratory shaker. The reaggregation of galactosidase treated cells (1) demonstrates a marked decrease in rate of adhesion compared to the control (C). Regression lines were generated from six points. Each point was the calculated average of three data values. (□) Galactosidase treated cells, (○) control cells.

Glycosidase Percent Aggregation Analysis

Three experimental data values for percent aggregation after 60 minutes of incubation were averaged together. They are exhibited in Table 9 along with their standard deviations. Percent decreases in aggregation were calculated which demonstrates the amount of inhibition occurring compared with the control. From these values, the enzymes were labeled in order of their capability to suppress re-aggregation.

Table 9 has displayed "t" values calculated by a two sample t-test. From this test one can find a significant difference between two mean values, in this case, an enzyme mean percent aggregation value and that of its control. The last column in Table 9 exhibits p values which do not support the hypothesis that there is a significant difference between the two means. This result is due to the large standard deviations involved and the small sample sizes.

Analysis of data acquired after 10, 20, 30, and 40 minutes of rotation showed that galactosidase inhibited aggregation to 57.7%, 23.2%, 36.7%, and 46.2% of the control value respectively. Galactosidase and glucosidase both occupied number one and two positions during the course of the experiment designating their relative effectiveness in inhibiting aggregation compared to the other

enzymes tested. Glucosidase treated cells exhibited decreased aggregation from control means of 61.4%, 74.0%, 39.6%, and 44.3% respectively. However, the contamination in this enzyme is relatively greater than galactosidase (see Materials). Therefore, the data presented on galactosidase may be a more accurate representation of its overall effectiveness.

Table 1. Data for regression lines produced by the loss of single cell number as a function of time. The best fitting regression was calculated by the BMD 05R program from six points, each point being the average of three data values for that particular time. X and Y mean indicate abscissa and ordinate means for each regression line. The intercept specifies the point in which the regression intercepts the ordinate. The regression coefficient (Reg Coeff) is the slope of the regression line followed by the standard error (Std error) which indicates the amount of variability of the slope due to the six non-linear points. The correlation coefficient (Corr Coeff) demonstrates the variability of the dependant variable, x with that of the independant variable, y. The significance of each correlation coefficient is indicated by asterisks, ** ($p < .01$), * ($p < .05$), and NS (not significant). The significance was also computed for the regression analysis (Reg Anal) between the regression coefficients of the control and saccharides at varying concentrations. The 95% confidence limit of the regression coefficient indicates the interval where the the regression coefficient has a significant probability of being located.

TABLE 1

REGRESSION DATA

Saccharide and Concentration	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff value n=6	Corr p value	p value Reg Anal df=1/12	95% confidence limit Reg Coeff ($\times 10^{-3}$)
Control	26.66667	6.09659	6.19826	-3.81	.28	-.98958	**	NS	
Mannose									
10 ⁻¹		6.13952	6.22776	-3.31	.29	-.98542	**	NS	0.80
10 ⁻²		6.11425	6.19599	-3.07	.46	-.95711	**	NS	1.27
10 ⁻³		6.11522	6.18164	-2.49	.44	-.94361	**	NS	1.22
10 ⁻⁴		6.10878	6.16954	-2.28	.58	-.89179	*	NS	1.67
10 ⁻⁵		6.10361	6.19305	-3.35	.36	-.97778	**	NS	0.99
Glucose									
10 ⁻¹		6.12155	6.19816	-2.87	.34	-.97302	**	NS	0.94
10 ⁻²		6.09123	6.17232	-3.04	.42	-.96334	**	NS	1.16
10 ⁻³		6.10180	6.18334	-3.06	.25	-.98696	**	NS	0.69
10 ⁻⁴		6.08733	6.16460	-2.90	.52	-.94066	**	NS	1.44
10 ⁻⁵		6.08144	6.15824	-2.88	.20	-.99062	**	NS	0.55
Galactose									
10 ⁻¹		6.09640	6.19054	-3.53	.37	-.97872	**	NS	1.02
10 ⁻²		6.11183	6.16704	-2.07	.32	-.95498	**	NS	0.88
10 ⁻³		6.09522	6.16274	-2.53	.05	-.99910	**	NS	0.13
10 ⁻⁴		6.09732	6.15657	-2.22	.36	-.95117	**	NS	0.99
10 ⁻⁵		6.09637	6.16134	-2.44	.35	-.96036	**	NS	0.97

TABLE 1 (con't)

REGRESSION DATA

Saccharide and Concentration	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff value n=6	Corr p value df=1/12	p Reg Anal	95% confidence limit Reg Coeff ($\times 10^{-3}$)
Control	26.66667	6.26337	6.34319	-2.99	.53	-.94213	**	NS	
Xylose									
10 ⁻¹		6.28074	6.35186	-2.67	.48	-.94099	**	NS	1.3
10 ⁻²		6.26818	6.34515	-2.89	.39	-.96512	**	NS	1.0
10 ⁻³		6.26593	6.33877	-2.73	.33	-.97150	**	NS	.9
10 ⁻⁴		6.27130	6.33537	-2.40	.57	-.90334	*	NS	1.5
10 ⁻⁵		6.25355	6.34097	-3.28	.49	-.95840	**	NS	1.3
Maltose									
10 ⁻¹		6.27867	6.36496	-3.24	.56	-.94553	**	NS	1.5
10 ⁻²		6.24428	6.33451	-3.38	.66	-.93172	**	NS	1.8
10 ⁻³		6.23733	6.32544	-3.30	.42	-.96961	**	NS	1.1
10 ⁻⁴		6.23763	6.33289	-3.57	.47	-.96686	**	NS	1.3
10 ⁻⁵		6.22665	6.31907	-3.47	.48	-.96301	**	NS	1.3
Stachyose									
10 ⁻¹		6.27949	6.35639	-2.88	.46	-.95241	**	NS	1.2
10 ⁻²		6.24373	6.32777	-3.15	.54	-.94683	**	NS	1.4
10 ⁻³		6.23854	6.30422	-2.46	.83	-.82945	**	NS	2.3
10 ⁻⁴		6.24939	6.31452	-2.78	.62	-.91238	**	NS	1.7
10 ⁻⁵		6.23330	6.30756	-2.78	.45	-.95198	**	NS	1.2

TABLE 1 (con't)

REGRESSION DATA

Saccharide and Concentration	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff n=6	Corr p value	p Reg Anal df=1/12	95% confidence limit Reg Coeff ($\times 10^{-3}$)
Control	26.66667	6.20619	6.30002	-3.52	.63	-.94160	**	NS	
Galacturonic acid									
10 ⁻¹		6.22418	6.30303	-2.96	.74	-.89341	*	NS	2.1
10 ⁻²		6.21006	6.28391	-2.77	.84	-.85510	*	NS	2.3
10 ⁻³		6.20831	6.27945	-2.67	.58	-.91786	**	NS	1.6
10 ⁻⁴		6.18509	6.28262	-3.66	.32	-.98489	**	NS	.8
10 ⁻⁵		6.16940	6.26496	-3.58	.45	-.97001	**	NS	1.2
Glucuronic acid									
10 ⁻¹		6.21278	6.30022	-3.28	.77	-.90540	*	NS	2.1
10 ⁻²		6.16701	6.25294	-3.22	.68	-.92105	**	NS	1.8
10 ⁻³		6.16706	6.24730	-3.01	.89	-.86187	*	NS	2.4
10 ⁻⁴		6.15838	6.24216	-3.14	.87	-.87575	*	NS	2.4
10 ⁻⁵		6.15389	6.22599	-2.70	.75	-.87412	*	NS	2.0
Sorbose									
10 ⁻¹		6.14534	6.22864	-3.12	.69	-.91520	**	NS	1.9
10 ⁻²		6.15617	6.23919	-3.11	.63	-.92776	**	NS	1.7
10 ⁻³		6.17148	6.24718	-2.84	.49	-.94609	**	NS	1.3
10 ⁻⁴		6.16639	6.23639	-2.62	.60	-.90902	*	NS	1.6
10 ⁻⁵		6.15880	6.22191	-2.37	.71	-.85602	*	NS	1.9

TABLE 1 (con't)

REGRESSION DATA

Saccharide and Concentration	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff n=6	Corr p value	p value Reg Anal df=1/12	95% confidence limit Reg Coeff ($\times 10^{-3}$)
Control	26.66667	6.27705	6.34622	-2.59	.53	-.92555	**	NS	
N-acetyl- mannosamine									
10 ⁻¹		6.31193	6.36784	-2.10	.40	-.93528	**	NS	1.1
10 ⁻²		6.29314	6.37530	-3.08	.44	-.96077	**	NS	1.2
10 ⁻³		6.29875	6.36969	-2.66	.52	-.93186	**	NS	1.4
10 ⁻⁴		6.30101	6.36179	-2.28	.55	-.90162	*	NS	1.5
10 ⁻⁵		6.28499	6.34243	-2.15	.43	-.92999	**	NS	1.1
N-acetyl- glucosamine									
10 ⁻¹		6.30678	6.37165	-2.43	.31	-.96920	**	NS	.8
10 ⁻²		6.28365	6.33657	-1.98	.29	-.95906	**	NS	.8
10 ⁻³		6.27581	6.33211	-2.11	.85	-.77836		NS	2.3
10 ⁻⁴		6.26476	6.31641	-1.94	.42	-.91908	**	NS	1.1
10 ⁻⁵		6.26715	6.32751	-2.26	.48	-.91954	**	NS	1.3
N-acetyl- galactosamine									
10 ⁻¹		6.27856	6.33836	-2.24	.33	-.95864	**	NS	.9
10 ⁻²		6.28341	6.33540	-1.95	.21	-.97772	**	NS	.5
10 ⁻³		6.28346	6.33614	-1.98	.44	-.91493	**	NS	1.2
10 ⁻⁴		6.26903	6.32165	-1.97	.39	-.92993	**	NS	1.0
10 ⁻⁵		6.26298	6.31439	-1.93	.29	-.95693	**	NS	.8

TABLE 1 (con't)

REGRESSION DATA

Saccharide and Concentration	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff value n=6	Corr p value	p value Reg Anal df=1/12	95% confidence limit Reg Coeff ($\times 10^{-3}$)
Control	26.66667	6.15943	6.30303	-5.39	.72	-.96578	**	NS	
Arabinose									
10 ⁻¹		6.18152	6.30593	-4.67	1.12	-.90169	*	NS	3.1
10 ⁻²		6.17430	6.30859	-5.04	1.01	-.92866	**	NS	2.8
10 ⁻³		6.16739	6.29237	-4.69	1.00	-.91972	**	NS	2.7
10 ⁻⁴		6.17221	6.30657	-5.04	1.18	-.90592	*	NS	3.2
10 ⁻⁵		6.16222	6.27171	-4.11	.95	-.90819	*	NS	2.6
Fructose									
10 ⁻¹		6.17065	6.29150	-4.53	.94	-.92315	**	NS	2.6
10 ⁻²		6.15861	6.27956	-4.54	.80	-.94273	**	NS	2.2
10 ⁻³		6.13885	6.25999	-4.54	.96	-.92040	**	NS	2.6
10 ⁻⁴		6.14106	6.24321	-3.83	.87	-.91014	**	NS	2.4
10 ⁻⁵		6.15594	6.25986	-3.90	.73	-.93722	**	NS	2.0
Fucose									
10 ⁻¹		6.14855	6.26348	-4.31	.80	-.93709	**	NS	2.2
10 ⁻²		6.16584	6.26507	-3.72	1.11	-.85833	*	NS	3.0
10 ⁻³		6.15778	6.26782	-4.13	.70	-.94692	**	NS	1.9
10 ⁻⁴		6.14199	6.24964	-4.04	.63	-.95518	**	NS	1.7
10 ⁻⁵		6.15347	6.26600	-4.22	.80	-.93490	**	NS	2.2

Table 2. Effects of Saccharides on rates of adhesion. Regression coefficients (Reg Coeff) or slopes indicate the rate of single cell loss due to adhesion. Regression coefficients for controls (times 10^{-3}) are shown as computed by the BMD 05R program. Mean values of the regression coefficients for a given sugar at its five different concentrations (10^{-1} to 10^{-5} M) were calculated and shown here with its standard deviation. Percent differences between regression coefficients of each sugar and its control were calculated and then the sugars were ordered (1 through 15) as to their ability to reduce the rate of adhesion.

TABLE 2

Effects of Saccharides on rates of adhesion

Saccharide	Reg Coeff ($\times 10^{-3}$)	Reg Coeff mean 10^{-1} - 10^{-5} M ($\times 10^{-3}$)	Standard deviation ($\times 10^{-3}$)	% difference from control	order
Control	-3.81				
Mannose		-2.90	.481	23.8	3
Glucose		-2.95	.092	22.5	4
Galactose		-2.55	.572	33.0	1
Control	-5.39				
Arabinose		-4.71	.380	12.6	10
Fructose		-4.26	.368	20.9	6
Fucose		-4.08	.227	24.3	2
Control	-2.59				
NA-Man		-2.45	.413	5.4	14
NA-Glu		-2.14	.203	17.3	8
NA-Gal		-2.04	.127	21.2	5
Control	-3.52				
Gal-uronic		-3.12	.461	11.3	11
Glu-uronic		-3.07	.230	12.7	9
Sorbose		-2.81	.322	20.2	7
Control	-2.99				
Xylose		-2.79	.324	6.6	12
Maltose		-3.39	.131	-13.3	15
Stachyose		-2.81	.247	6.0	13

Table 3. Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryo cells after 10 minutes of rotation. The mean percent aggregation was calculated from 15 data values for a given sugar ranging in concentration from 10^{-1} to 10^{-5} M and shown here along with its standard deviation. The percent difference between the mean percent aggregation of the sugar and control were calculated and from this, the sugars were then ordered as to its ability to inhibit aggregation. "t" values were computed to reveal if the differences between sugar and control means were significant. Asterisks indicate significance of the two-sample t-test ** ($p < .01$), * ($p < .05$), and NS (not significant).

Table 4. Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryo cells after 20 minutes of rotation. (see legend of table 3)

Table 5. Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryo cells after 30 minutes of rotation. (see legend of table 3)

Table 6. Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryo cells after 40 minutes of rotation. (see legend of table 3)

Table 7. Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryo cells after 60 minutes of rotation. (see legend of table 3)

TABLE 3

Effects of Saccharides on percent aggregation (agg) of dissociated 24 hour sea urchin embryos after 10 minutes of rotation.

Saccharide	mean % agg	Standard deviation	% difference from control	order	t value	p value (n+n=30)
Control	16.2	5.7				
Mannose	10.1	7.3	37.7	6	2.55	*
Glucose	7.8	8.1	51.9	4	3.28	**
Galactose	6.6	8.9	59.3	2	3.52	**
Arabinose	18.0	6.0	-11.1	15	.84	NS
Fructose	15.5	9.6	4.3	12	-.24	NS
Fucose	13.1	13.8	19.1	8	.80	NS
NA-Man	9.6	6.7	40.7	5	2.91	**
NA-Glu	7.7	4.2	52.5	3	6.51	**
NA-Gal	4.3	7.5	73.5	1	4.85	**
Gal-uronic	13.5	4.3	16.7	10	1.46	
Glu-uronic	15.6	5.7	3.7	13	.29	NS
Sorbose	16.8	4.1	-3.7	14	.33	NS
Xylose	11.8	3.1	27.2	7	2.60	*
Maltose	15.4	3.7	4.9	11	.46	NS
Stachyose	13.5	4.8	16.7	9	1.40	

TABLE 4

Effects of Saccharides on percent aggregation (agg) of dissociated
24 hour sea urchin embryos after 20 minutes of rotation.

Saccharide	mean % agg	Standard deviation	% difference from control	order	t value	p value (n+n=30)
Control	23.5	7.7				
Mannose	15.8	8.2	32.8	4	2.65	*
Glucose	16.0	10.2	31.9	5	2.27	*
Galactose	10.9	5.6	53.6	2	5.13	**
Arabinose	32.3	9.9	-37.4	15	2.72	**
Fructose	29.5	9.9	-25.5	14	1.85	
Fucose	28.2	10.7	-20.0	13	1.38	
NA-Man	17.8	6.5	24.3	6	2.19	*
NA-Glu	14.3	8.0	39.1	3	3.21	**
NA-Gal	10.7	7.7	54.5	1	4.55	**
Gal-uronic	21.8	3.7	7.2	9	.77	NS
Glu-uronic	24.4	8.1	-3.8	12	.31	NS
Sorbose	23.8	4.4	-1.3	10	.13	NS
Xylose	19.4	3.9	17.4	7	1.84	
Maltose	23.9	3.1	-1.7	11	.19	NS
Stachyose	21.2	4.1	9.8	8	1.02	NS

TABLE 5

Effects of Saccharides on percent aggregation (agg) of dissociated
24 hour sea urchin embryos after 30 minutes of rotation.

Saccharide	mean % agg	Standard deviation	% difference from control	order	t value	p value (n+n=30)
Control	29.2	10.6				
Mannose	22.0	10.5	24.7	5	1.87	
Glucose	21.7	8.7	25.7	4	2.12	*
Galactose	16.6	9.5	43.2	1	3.43	**
Arabinose	43.0	5.6	-47.3	15	4.46	**
Fructose	37.3	6.1	-27.7	14	2.57	*
Fucose	35.3	9.8	-20.9	13	1.64	
NA-Man	23.7	7.6	18.8	6	1.63	
NA-Glu	20.2	11.1	30.8	3	2.27	*
NA-Gal	17.3	7.1	40.8	2	3.61	**
Gal-uronic	26.8	5.6	8.2	9	.78	NS
Glu-uronic	32.3	7.4	-10.6	12	.93	NS
Sorbose	28.4	5.8	-2.7	10	.26	NS
Xylose	25.7	6.1	12.0	7	1.11	NS
Maltose	29.1	4.7	.3	11	.03	NS
Stachyose	26.8	4.0	8.2	8	.82	NS

TABLE 6

Effects of Saccharides on percent aggregation (agg) of dissociated
24 hour sea urchin embryos after 40 minutes of rotation.

Saccharide	mean % agg	Standard deviation	% difference from control	order	t value	p value (n+n=30)
Control	35.1	8.3				
Mannose	28.6	10.1	18.5	7	1.93	
Glucose	25.9	10.7	26.2	4	2.63	*
Galactose	22.8	11.5	35.0	2	3.36	**
Arabinose	45.1	5.2	-28.5	15	3.95	**
Fructose	41.2	7.2	-17.4	14	2.15	*
Fucose	37.2	5.0	- 6.0	13	.84	NS
NA-Man	27.8	9.1	20.8	5	2.30	*
NA-Glu	23.0	9.0	34.5	3	3.83	**
NA-Gal	20.3	7.5	42.2	1	5.12	**
Gal-uronic	33.1	5.5	5.7	11	.78	NS
Glu-uronic	33.0	8.9	6.0	10	.67	NS
Sorbose	29.8	6.7	15.1	8	1.92	
Xylose	28.1	5.5	19.9	6	2.72	**
Maltose	33.1	5.0	5.7	12	.80	NS
Stachyose	30.7	4.2	12.5	9	1.83	

TABLE 7

Effects of Saccharides on percent aggregation (agg) of dissociated
24 hour sea urchin embryos after 60 minutes of rotation.

Saccharides	mean % agg	Standard deviation	% difference from control	order	t value	p value (n+n=30)
Control	40.1	11.2				
Mannose	30.7	13.2	23.4	5	2.10	*
Glucose	31.3	10.8	21.9	6	2.30	*
Galactose	25.5	11.4	36.4	3	3.50	**
Arabinose	47.0	4.7	-17.2	15	2.20	*
Fructose	44.1	6.4	-10.0	14	1.20	NS
Fucose	42.7	3.9	- 6.5	13	.84	NS
NA-Man	29.8	8.7	25.7	4	4.50	**
NA-Glu	24.3	10.6	39.4	2	3.90	**
NA-Gal	21.8	7.8	45.6	1	5.10	**
Gal-uronic	34.8	8.0	13.2	9	1.40	
Glu-uronic	34.8	9.0	13.2	10	1.40	
Sorbose	34.8	4.6	13.2	11	1.60	
Xylose	32.0	5.3	20.2	7	2.59	*
Maltose	38.8	8.0	3.2	12	.36	
Stachyose	32.1	4.1	20.0	8	2.60	*

Table 8. Data for regression lines produced by the loss of single cell number as a function of time. The best fitting regression was calculated by the BMD 05R program from six points, each point being the average of three data values for that particular time. X and Y mean indicate abscissa and ordinate means for each regression line. The intercept specifies the point in which the regression intercepts the ordinate. The regression coefficient (Reg Coeff) is the slope of the regression line followed by the standard error (Std error) which indicates the amount of variability of the slope due to the six non-linear points. The correlation coefficient (Corr Coeff) demonstrates the variability of the dependant variable, x with that of the independant variable, y. The significance of each correlation coefficient is indicated by asterisks, ** ($p < .01$), * ($p < .05$), and NS (not significant). The significance was also computed for the regression analysis (Reg Anal) between the regression coefficients of the control and the glycosidase.

TABLE 8

GLYCOSIDASE REGRESSION DATA

Enzyme and Control	X mean	Y mean	Intercept	Reg Coeff ($\times 10^{-3}$)	Std error Reg Coeff ($\times 10^{-3}$)	Corr Coeff	Corr Coeff p value n=6	p value Reg Anal df=1/12
Fucosidase	26.66667	6.28792	6.35329	-2.45	.50	-.92692	**	NS
Control		6.26236	6.31376	-1.93	.46	-.90239	*	NS
Mannosidase		6.30551	6.43267	-4.77	.50	-.97862	**	NS
Control		6.30407	6.39652	-3.47	.74	-.92006	**	NS
NA-glucos- amine		6.31568	6.40150	-3.22	.82	-.89121	*	NS
Control		6.30162	6.42565	-4.65	.53	-.97542	**	NS
Glucosidase		6.27805	6.33147	-2.00	.48	-.90039	*	NS
Control		6.25749	6.35039	-3.48	.37	-.97819	**	NS
Galactosidase		6.33911	6.36196	-0.86	.43	-.70459		NS
Control		6.29939	6.36931	-2.62	.42	-.95320	**	NS

Table 9. Effects of glycosidase on rates of adhesion and percent aggregation after 60 minutes of rotation. Regression coefficients were computed from six points (each point being the average of three data points) by the BMD 05R program. Percent differences were calculated for each glycosidase and its individual control and then the glycosidase were ordered according to its ability to reduce the rate of adhesion.

The percent aggregation (% agg) for dissociated 24 hour sea urchin cells after 60 minutes of rotation is displayed in column 5. Each value was the average of three experiments therefore, the standard deviation is given alongside the mean. Percent differences were calculated between each glycosidase and its control and then each glycosidase was ordered as to its effects on inhibiting reaggregation. A two sample t-test was utilized to reveal significant differences between glycosidase and control values. Significance was indicated by asterisks ** ($p < .01$), * ($p < .05$), and NS ($p > .75$).

TABLE 9

Effects of glycosidase on rates of adhesion
and % aggregation after 60 minutes of rotation

Enzyme and Control	Reg. Coeff. ($\times 10^{-3}$)	% difference from control	order	% agg	Standard deviation	difference from control	t value	p value (n+n=6)	order
Fucosidase Control	-2.45 -1.93	-26.9	4	33.1 21.6	13.7 7.3	-53.2	1.048	-	5
Mannosidase Control	-4.77 -3.47	-37.4	5	39.9 40.6	1.1 4.3	1.7	.222	-	4
NA-glucose- amine Control	-3.22 -4.65	30.7	3	33.5 46.4	12.6 8.7	27.8	.379	-	3
Glucosidase Control	-2.00 -3.48	42.5	2	17.2 37.6	14.8 2.9	54.2	1.912	-	2
Galacto- sidase Control	-0.86 -2.62	67.1	1	12.7 33.3	7.3 5.9	61.9	3.106	-	1

DISCUSSION

What causes the reaggregation of the sea urchin embryo cells to one another? Initially, the attractive force between two cells may be the Van der Waals interaction (Nir and Anderson, 1977). The Van der Waals attraction is created by charged particles on the cell surface such as proteins, sugars, phospholipids, and cholesterol which create polarized environments immediately surrounding the cells. Nir and Anderson have formally investigated the impact of each of these membrane components on the attractive forces between cell surfaces and have found that changes in the ratio of particular polar molecules on the membrane surface and media can alter the adhesive bond between cells. There is no indication that this could be the only adhesive force however, unless perhaps the ratio of membrane components is responsible for the specificity of cell types. Bell (1978) has characterized the electrical forces as non-specific but suggests that they could enable specific bonds to form between similar cells.

The experimentation performed in this study suggested that specific carbohydrates are important components in the adhesion of dissociated sea urchin embryo cells. The two

sugars, N-acetyl-galactosamine and galactose, consistently inhibited the reaggregation to the greatest extent at all time points (10, 20, 30, 40, and 60 minutes) observed after rotation. The differences from control values were significant to the $p < .01$ level as determined by the two sample t-test. N-acetyl-glucosamine and glucose ranked third and fourth among the 15 sugars at all time points which indicated a significant difference ($p < .05$). All other sugars showed no consistent behavior.

Regression analysis, created by monitoring the loss of single cells as a function of time, confirmed the above finding by producing regression lines for N-acetyl-galactosamine and galactose with a lower slope than its control. The differences among the slopes indicate a lower rate of adhesion.

The glycosidases tested showed galactosidase to be effective in inhibiting reaggregation at each time point although not to a significant extent ($p > .05$). The slope of its regression line displayed a greatly decreased value.

These two observations, in conjunction, implicate galactopyranosyl-like groups as the specific saccharide involved in the binding of the sea urchin membranes. Comparing the relative effectiveness of the fifteen sugars, it appears that the C5 substituent group is an important portion of the sugar (see Figure 3). If the primary alcohol of galactose is oxidized as in D-galacturonic acid, reduced

X = -CH ₂ OH	D-Galactose
X = -H	L-Arabinose
X = -CH ₃	D-Fucose
X = -COOH	D-Galacturonic acid

Figure 3. The configuration of some monosaccharides tested in the hapten inhibition studies.

as in D-fucose, or absent as in L-arabinose, the efficiency as an inhibitor is reduced. Orientation around the C⁴ seems to make a slight difference but since glucose and N-acetyl-glucosamine were significant inhibitors it appears that the change is not as detrimental (see Figure 4). The acetylated amino group on C² also shows no critical advantage to the inhibitor.

Maltose (two linked glucose molecules) and stachyose (α -D-galactosyl- α -D-galactosyl- α -D-glucosyl- β -D-fructose) are not potent inhibitors. This can imply that either the specificity for the inhibitor must include the penultimate residue which is not correct in both saccharides or that the determinant structure does not require that a sugar be linked to another sugar for adhesion to occur. The galactopyranosyl-like group may, for example, be attached directly to a protein. This explanation has more credibility in light of the well known O-glycosidic linkages between N-acetyl-galactosamine and hydroxyl groups of serine and threonine in many glycoproteins (Thomas and Winzler, 1969; Bahl et al., 1972; Baenziger and Kornfeld, 1974; Kieras, 1974; Spiro and Bhoyroo, 1974; Codington et al., 1975; Shier, 1975; Newman et al., 1976).

A variety of laboratories have identified galactose residues on membrane glycoproteins. Galactose has been shown to exist on Ehrlich ascites carcinoma cells (Eylar and Cook, 1965), Chinese hamster ovary (Juliano and Li,

D-galactose

D-glucose

D-mannose

Figure 4. The configuration of some monosaccharides tested in the hapten inhibition studies.

1978), pig lymphocytes (Newman et al., 1976), and human erythrocytes (Thomas et al., 1969). Glycolipids have also been shown to contain galactose within membranes (Cuatrecasas, 1973). More importantly, galactose has been shown to be the terminal residue of oligosaccharide chains among many different cell types through chemical analysis, including TA3-Ha murine mammary carcinoma ascites cells (Codington et al., 1975), calf thymocytes (Kornfeld, 1978), and as determinants for type A antigens on human erythrocytes (Ginsburg et al., 1971). Terminal galactose identification has been accomplished through lectin studies. Lectins which are known to bind galactose residues have been used to demonstrate the presence of terminal galactose units in a variety of systems such as a lectin from the mushroom Agaricus bisporus binding to calf thymocyte membranes (Kornfeld, 1978), Ricinus communis binding to normal 3T3 and transformed SV3T3 and 3T12 cells (Nicolson and Lacorbiere, 1973), peanut agglutinin binding to embryonal carcinoma cells (Reisner et al., 1977), lectins from the slime mold Dictyostelium discoideum binding to formalized rabbit erythrocytes (Rosen et al., 1975; Frazier et al., 1975), formalized sheep erythrocytes (Simpson et al., 1974; Frazier et al., 1975; Barondes et al., 1974), formalized human type O erythrocytes (Frazier et al., 1975; Simpson et al., 1975), and fixed cohesive Dictyostelium discoideum cells (Reitherman et al., 1975). Evidence has shown N-

acetyl-galactosamine to exist as terminal residues on type A blood cells (Kabat, 1956; Landsteiner, 1962; Ginsburg et al., 1971; Kornfeld et al., 1971; Sharon and Lis, 1972; Reitherman, 1974), formalized sheep erythrocytes (Rosen et al., 1973), and formalized rabbit erythrocytes (Rosen et al., 1975).

The specificity of the terminal sugars on sea urchin cell surfaces could enable recognition among each other during development. During gastrulation, all movement and cell contacts could be determined by the cell membrane (Gustafson and Wolpert, 1967). M^CClay and Chambers (1978) have shown the appearance of paternal antigens on hybrid sea urchin embryo cells during gastrulation. These researchers have also demonstrated a species specific adhesion to paternal type aggregates after gastrulation by the hybrid cells (M^CClay, Chambers, and Warren, 1977) which can be blocked by covering the paternal antigens with univalent Fab antibody fragments. They suggest that these particular antigens mediate the recognition and adhesion of the sea urchin cells. Although the particular sea urchin antigens have not been characterized as to their composition, similar experiments have been performed covering carbohydrate containing antigens with univalent antibodies resulting in alterations in contact phenomena in tumor and slime mold cells (Beug et al., 1970; Burger and Noonan, 1970).

This study utilized sea urchin embryo cells that were

allowed to develop 24 hours before being dissociated into a single cell suspension. This is the approximate time when gastrulation occurs. It has been suggested by various researchers that surface heterosaccharides can aid in many cellular processes (Ginsburg et al., 1971; Sugiyama, 1972; Cook and Stoddart, 1973). These cellular processes may include the mechanisms by which gastrulation is mediated. During gastrulation a great deal of cell movement takes place which would dictate the necessity for recognition and intra-organismal specific adhesion to occur between cells (Steinberg, 1963; Gustafson and Wolpert, 1967). Whether N-acetyl-galactosamine and galactose are terminal sugars that provide for the adhesive requirements unique to gastrulation is unknown. However, the conclusion that N-acetyl-galactosamine and galactose residues partially assist in sea urchin gastrulation is suggested.

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