A Quantitative Evaluation of the Relative Cohesive Strengths of Specific Cell Types

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A QUANTITATIVE EVALUATION OF THE RELATIVE COHESIVE STRENGTHS OF SPECIFIC CELL TYPES

The major portion of the following report is the first draft of a project summary submitted for publication in "Nature" magazine in October, 1969. The same project was continued under the Strnad Fellowship Program with stipulated changes. The continuation was mainly an attempt to accumulate more data on the cohesive strengths of heart and neural retina cells of embryonic chicks.

Abstract

In 1963, Malcom S. Steinberg developed the hypothesis that some morphogenetic tissue movements follow patterns resulting directly from laws of thermodynamic equilibrium. The hypothesis states that cell re-aggregation results from the relationship between the cohesive strength of each cell type and the strength of adhesive forces between the different cell types.

While there is experimental evidence that this hypothesis is correct, there also exists the possibility that tissues migrate in a manner that exactly contradicts the laws of thermodynamic equilibrium. For example, Steinberg's hypothesis states that in an aggregate containing two cell types, the more cohesive cells move toward the center of the aggregate, leaving a minimum of surface free energy in the aggregate. If the true tendency of cells were to have a maximum of surface free energy, the least cohesive cells would move toward the center, and basically the same phenomenon would be observed. It is therefore necessary to determine quantitatively the relative strengths of the cohesive forces within a homogeneous cell aggregate (work of cohesion) of each cell type studied.

Introduction

Steinberg's hypothesis states that the condition determining the thermodynamic equilibrium of a system containing two mutually adhesive mobile systems is that the overall system have a minimum of surface free energy. A minimum surface free energy in turn requires a minimum surface area (as achieved in a

sphere) and a minimum amount of energy per unit area. The adhesions which are weaker give a lower surface energy than stronger adhesions. This means that the relationship between the works of cohesion (cohesive strength of like cell types) of two systems "a" and "b" and the work of adhesion (strength of adhesion of unlike cell types) between those systems determines the pattern which results in thermodynamic equilibrium. The purpose of this experiment is to measure the works of cohesion and work of adhesion between specific cell types.

Materials and Methods

Heart cells of five-day-old chick embryos and neural retina cells of seven-day-old embryos were obtained by dissecting the embryos in a calcium-magnesium-free saline solution in a Petri dish.

In August, 1969, in vivo cell concentrations were determined by fixing cells immediately after removal from embryos in Bouin's fixative for about 20 hours. The tissues were sectioned at 7 microns and stained with Harris' Haematoxylin and Eosin stain. The average cell separation was determined by counting the number of uniformly aligned cells in a single unit on a calibrated grid at a magnification of 540x. The number of cells in a 3x3 unit area on the same grid was determined and the depth of the cell layer was assumed to be the cell separation. The cell concentration per milliliter was determined from the cell concentration per measured volume.

During the dissection procedure (during the Strnad Fellowship Program) the cells remained in saline solution for about 40 minutes. The surface area and volume of the tissues was determined by placing the Petri dish on a stereoscopic dissecting microscope with a grid eye-piece calibrated for a magnification of 18x. Liver and heart tissues were assumed to be generally spherical, while neural retina tissues (which roll up after removal from the eye) were assumed to be cylindrical.

After determining the dimensions of tissues, they were transferred to numbered 25 ml Erlenmeyer flasks containing 3 ml of a solution of .25% trypsin, 0.1% DNase, and 0.5% collagenase (% in g/liter) the balance being calcium-magnesium-free saline.

The flasks were placed on a shaker in an incubator room at 37.5°C and gyrated at about 75-100 gyrations per minute. The tissues remained in the flasks for a total of 30 minutes. They were then transferred to centrifuge tubes and mixed with a vortex mixer for 30 seconds.

Medium (from the bottom of the tube) was placed in a counting chamber and the cell concentration per milliliter was determined.

Results

Data tables show the work of cohesion calculations determined during the project. The data indicates that heart cells are the more cohesive type. Aggregates containing binary combinations with neural retinal cells should follow case 2 under "Discussion."

Discussion

The possible relationships that may exist between the relative works of cohesion and work of adhesion are: (1) that the work of adhesion is greater than or equal to the average of the works of cohesion, (2) that the work of adhesion is less than the average of the works of cohesion, but greater than one of the works of cohesion, or (3) that the work of adhesion is less than either of the works of cohesion, and therefore less than the average of the works of cohesion.

If system "a" is defined as the more cohesive of two systems, and system "b" the less cohesive, the preceeding conditions are given by the equations: (1) W_{ab} - ½ (W_a + W_b) and (2) ½(W_a + W_b). W_{ab} where W_a is the work of cohesion of system "a," W_b the work of cohesion of "b," and W_{ab} the work of adhesion between the two systems. Equation 2 implies that either (3) W_{ab} W_b or (4) W_{ab} W_b. Steinberg deduced that case 1 (equation 1) will give a final equilibrium system with "a" and "b" adhesions alternately arranged. Case 2 (equations 2 and 3) should result in a system with "a" adhesions enclosed by "b" adhesions. Case 3 (equations 2 and 4) will result in two segregated systems with the degree of segregation being dependent upon the weakness of the work of adhesion.[3]

Because the work of adhesion can be calculated from W_a and W_b[4] it is necessary only to measure quantitatively the relative work of cohesion of each system under consideration.

Quantitative evaluation of the work of cohesion of a cell type was defined in arbitrary units using the definition:

$$W_c = 10 \log 10 \frac{\text{in vivo cell conc. x no. of tissues used x k}}{\text{cell concentration after dissociation}}$$

$$\frac{\text{x av. vol. of tissue}}{\text{x vol. of media}}$$

where "k" is a proportionality constant equal to the ratio of the in vivo cell concentration ("natural cell concentration") of one cell type to a single cell type used as a reference for all other types used. It is assumed that if two cell types dissociate under a given set of environmental conditions with most variables constant, then the degree of dissociation under those conditions is a result of the nature of the adhesive properties of each cell type. It follows, then, that a cell type "a" which dissociates more in trypsin than a type "b" has weaker cohesive forces in trypsin than does cell type "b." The work of cohesion is therefore inversely proportional to the cell concentration after dissociation. The degree of dissociation is also affected by the number of tissues used. If more tissues are dissociated, more cells are available, and the "dissociation concentration" (concentration of cells after dissociation) would be higher, making the work of cohesion appear lower. The work of cohesion is therefore directly proportional to the ratio of the number of tissues to the final dissociation concentration.

The number and concentration of cells available for dissociation depends on the volume of the tissues dissociated, the natural concentration of cells in the tissues, and the volume of the medium in the flask. The number of cells available is equal to the natural concentration in cells/unit volume times the volume of the tissue (V_t). When this number is divided by the volume of the medium in the flask (V_f), the highest possible concentration of cells in the flask is obtained. The work of cohesion is then defined as the ratio between the possible cell concentration in the flask and the actual cell concentration after dissociation.

For two cell types differing significantly in size and shape, dissociation will be affected in that differing percentages of each cell surface will be affected by trypsin and because cells with a high in vivo concentration which do not dissociate in proportion to that concentration will appear to be more cohesive than they actually are. The equation would therefore be more likely to be valid if the cell types used have the same in vivo concentration. The in vivo concentration is therefore "adjusted" with reference to a particular cell type using the ratio of the concentration of the cell types. This ratio gives the effect of "mathematically stretching" smaller cells to the size of larger, elongated cells.

If in a hypothetical situation, there were almost no cohesive forces in a system, the work of cohesion using the factors discussed in preceeding paragraphs would equal 1. It would seem natural that if there is no work of cohesion, the work of cohesion is 0. Therefore, the common logorithm of the calculated value is used, and multiplied by 10 so that differences in values are more apparent.

If the in vivo cell concentration is represented as c_1, the dissociation concentration is c_2, n is the number of tissues used, and k is the proportionality constant, the work of cohesion using a trypsin dissociation is given as:

$$W_c = 10 \log \frac{c_1 \; k \; n \; v_1}{c_2 \; vt}$$

Conclusion

A trypsin dissociation technique has been found which gives relative values for the work of cohesion consistent with Steinberg's hypothesis that cells re-aggregate according to the laws of thermodynamic equilibrium. It may be used to predict patterns of cell aggregations at equilibrium.

Variations in Techniques

For practical reasons, the following changes in techniques were necessary during the Strnad Fellowship project. (The original techniques were used from July to August, 1969 at University Hospitals.)

1. 50 ml Erlenmeyer flasks were used in place of 25 ml flasks.
2. The actual trypsin concentration was .025% instead of .25% as a result

of a purchasing error.

3. All flasks were agitated at room temperature because no incubators large enough to accomodate the shaker were available.

Changes in Results

The data collected as a part of the Strnad Fellowship appears on the following page. Because of the change in techniques, it could not be correlated with data collected previously.

Work of Cohesion

The data collected during the Strnad Fellowship contradicts its theoretical limits inasmuch as the ratio of concentrations before and after dissociation should not be less than one. The only conclusion available is that one of the measurements used is incorrect by at least one order of magnitude. Because extensive investigation is required to find the error, the definition of the work of cohesion is now simply the ratio of the two observed concentrations, and not a factor of the logarithm of that ratio. Note also that neural retina cells were used as the "reference" type mentioned on the last page.

Results of Strnad Fellowship Project

Data Unit No.	Observed volume x 10^{-6} cc	clnkv- c_2 v f
Neural Retina		
1	4.9	.46
2	9.1	.80
3	4.2	.68
4	8.3	.82
5	8.3	1.1
6	20.	.32
7	15.	1.1
8	40.	1.5
9	15.	.66
10	11.	.16
11	19.3	.15
12	10.	.50
13	15.	.31
14	10.	.46
15	41.	.99
16	23.	.56
17	26.	.64
18	34.	.83
19	39.	.94
20	77.	.37
	Mean	.65
Heart	x10^{-3}	x10^{4}
1	6.0	6.9
2	1.3	1.9
3	2.1	4.0
4	1.2	2.1
5	.38	.07?
6	1.6	4.7
7	.95	2.8
8	.82	4.7
9	1.6	.95
10	.57	3.3
11	.76	4.3
12	1.0	2.9
13	.17	.24
14	3.0	.884
15	1.8	2.1
16	67.	.78
17	.87	.72
18	.59	.86
19	.72	1.4
20	.12	.09?
	Mean	2.37

References

1. Steinberg, M. S. 1965. Reconstruction of Tissues by Dissociated Cells, p. 67-74 in Bell, Eugene, Molecular and Cellular Aspects of Cell Development, Harper and Row, New York, Evansten, and London.

2. Steinberg, M. S. 1962. On the Mechanism of Tissue Reconstruction by Dissociated Cells, I. Population Kinetics, Differential Adhesiveness, and the Absence of Directed Migration, Proc. N.A.S. (U.S.), 48, 1577-1582.