CELL SORTING OUT: THE SELF-ASSEMBLY OF TISSUES *IN VITRO*

Author: **Peter B. Armstrong**
Department of Zoology
University of California
Davis, California

Referee: Malcolm S. Steinberg
Department of Biology
Princeton University
Princeton, New Jersey

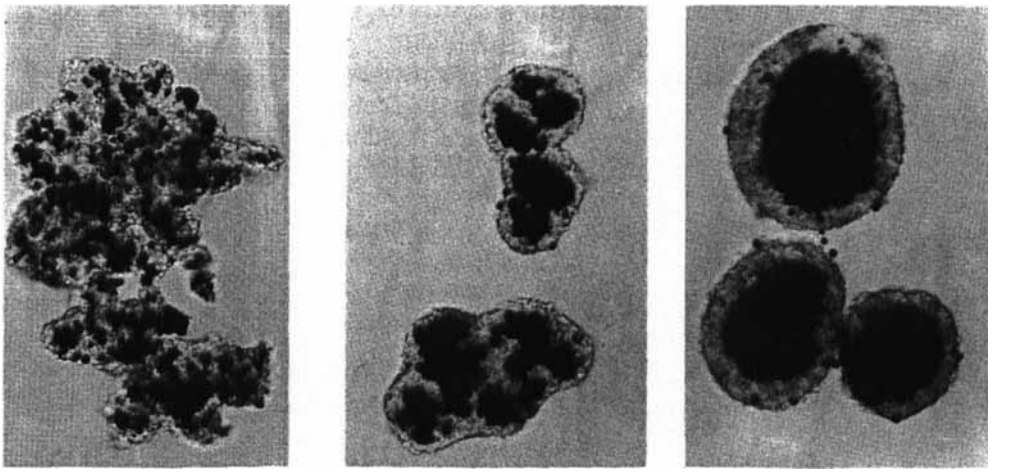
I. INTRODUCTION

The sciences of anatomy and histology present the picture of living organisms as precisely organized ensembles of cells, tissues, and organs. Understanding the factors that produce tissue organization during development is one of the fundamental goals of experimental biology. What is desired is an accounting of tissue organization based on properties and behavior of the constituent cells and molecules. Two complementary stratagems have been devised to explore this problem. In one, the investigator attempts to provide an understanding of the processes that govern the development of tissue placement during embryonic morphogenesis. This includes study of the factors that determine the pathways migratory cells and tissues follow during the morphogenetic movements which play very important roles in establishing the final patterning of tissues.[1-4] This stratagem seeks to account for tissue organization from an understanding of the developmental history of the particular structure of interest.

The second stratagem involves experimental perturbation of the organization of the fully formed organ followed by determination of the capacity for reestablishment of a normal pattern. This second method of analysis has the potential to provide information on the nature of both the processes that establish the definitive arrangements of tissues brought into apposition by cell movement and the processes that stabilize and maintain tissue structure thereafter. The opportunities for experimental perturbation of tissue organization with the goal of elucidating the underlying principles governing that organization include wounding and amputation experiments *in vivo*[5] and a variety of studies that can be conducted *in vitro*. An example of the latter is provided by studies of cell sorting of organ-cultured cell aggregates comprised initially of cohering, randomly intermingled populations of two or more cell types (Figure 1). Typically, sorting out results in the establishment of homogeneous tissue domains that remain adherent in a patterned array characteristic for the cell types employed. If the cells are taken from tissues that are normally in association, the final pattern often bears a striking resemblance to the organization of the tissues *in situ*. The present article reviews studies on cell sorting and allied experiments and attempts to show how such studies contribute to our understanding of tissue organization at the cellular and biochemical levels.

II. SELF-ORGANIZATION BY ORGAN-CULTURED CELL AGGREGATES: CELL SORTING AND RELATED PHENOMENA

Cell sorting is the process by which cohering, disorganized aggregates of cells establish structured tissues. In many situations of cell sorting, the aggregates contain two or more different cell types. Sorting in these cases transforms an initially disordered array of cohering



A B C

FIGURE 1. Living aggregates of 7-d-old chick embryo neural retina (unpigmented cells) and pigmented retina (darkly pigmented cells) maintained in stirred suspension culture. (A) 5-H aggregate: the two cell types are mixed in a disordered fashion in the aggregate; (B) 19-h aggregate: pigmented retinal cells have vacated the surfaces of the aggregates and are found in numerous irregular-shaped internal clumps embedded in neural retinal tissue; and (C) 2-d aggregates: fusion of internal pigmented retinal cell clumps has segregated the majority of these cells into a single internal mass which is completely enveloped by neural tissue. The scattered pigmented retinal cells at the surfaces of the aggregates are probably moribund cells in the process of sloughing.

Table 1
RECONSTRUCTION OF NORMAL HISTOLOGY OF VERTEBRATE ORGANS BY CELL SORTING

Organ	Reconstructed structure	Ref.
Skin	Feather follicles that produce feathers	16—20
Otocyst	Vesicles of sensory epithelium surrounded by connective tissue	21
Brain	Central cellular layer, peripheral molecular layer, parallel oriented pyramidal neurons in curved plate (Ammon's horn), segregation of immature and mature neurons, radial orientation of astroglia, clustering of specific types of neurons, surface ependyma	22—28
Limb bud	Myoblasts surround chondroblasts	29, 30
Pronephros	Pronephric duct	6
Mesonephros	Outer capsule, nephric tubules embedded in connective tissue	30—33
Metanephros	Outer capsule, inner pelvis-like cavity, secretory and collecting tubules	20, 34, 35
Testis	Tubules of Sertoli cells surrounding germ cells and embedded in connective tissue	36—38
Ovary	Interior nests of germ cells surrounded by follicular envelope, external germinal epithelium	39, 40
Thyroid	Epithelial follicles embedded in mesenchyme	41—47
Lung	Alveoli lined by pneumocysts, branched interconnected tubules embedded in mesenchyme	48—52
Liver	Outer capsule, hepatocyte cords with central bile canaliculi, connective tissue cortex, hematopoietic islands in parenchyma	20, 53—55

cells into one in which the cells are organized into homogeneous tissue domains. First described for organ-cultured aggregates of amphibian embryo cells,[6] sorting has been demonstrated for mixed populations of cells from a variety of phylogenetic groups including invertebrates[7-15] and vertebrates (Table 1), in both cell aggregates (Table 1) and in monolayer culture,[56-58] and with cells from embryonic (Table 1), postnatal,[38,40,55] and adult[41,45,54,59]

stages of development. Figure 1, which depicts stages in the sorting of chick embryonic neural retinal and pigmented retinal cells in organ-cultured aggregates, illustrates the process. The initial aggregate produced by reaggregation of suspensions of dissociated cells of the two cell types contains both cells in a disordered array. This is replaced during a period of 1 to 2 d in organ culture by one in which the cells are organized into relatively homogeneous tissue domains. Typically, the homogeneous domains are positioned with respect to each other in a characteristic and reproducible relationship that is characteristic for the cell types included in the aggregate. In the case illustrated in Figure 1, a majority of the pigmented retinal cells occupy a domain in the center of the aggregate which is surrounded completely by a superficial layer of neural retinal tissue. This arrangement, with one tissue occupying the center and surrounded completely by the second tissue, is the most commonly reported consequence of sorting out of binary aggregates.[60]

Several general points can be made about cell sorting out: (1) cell sorting out, by definition, is the process by which cohering, disorganized cell populations establish homogeneous tissues; (2) cell sorting can occur with mixed cell populations whether the cells are from tissues that normally are in contact or are from tissues that *in situ* are not associated; (3) the patterned array generated by cell sorting usually is reproducible for a given pairing of cell types; and (4) if the cell types combined in a sorting experiment in organ culture are from tissues normally in association *in vivo*, then the final organization frequently bears a striking similarity to the organization of those two tissues *in vivo* (Table 1). Item 3 above warrants special note. One goal of any attempt to explain cell sorting must include an accounting, not only for the establishment of tissue homogeneity, but also for the reproducible patterning of the tissues that result. The ability of disordered cell aggregates to restore normal tissue architecture (point 4 above) suggests that an understanding of the mechanisms underlying cell sorting *in vivo* should prove instructive in understanding the processes that govern and stabilize the definitive relationships of the tissues associated with each other in the various organs of the body. In addition to the reestablishment of normal histotypic relationships (Table 1), cell sorting can result in the recovery of normal patterns of cell junctions[61] and in the recovery of the functional and biochemical characteristics of the parent organ.[62-64] Thus, although the events of cell sorting do not mimic the pathways of the morphogenic movements which are so important in establishing tissue associations (during normal morphogenesis, the tissues of individual organs do not sort out into their final form from random mixtures of the constituent cells), an understanding of the mechanisms by which cultured heterotypic cell aggregates generate patterned arrays of tissues shows promise for analyzing the processes that the embryo employs to produce the definitive organization of tissues brought into association by prior morphogenetic cell movement.

Analysis of the mechanisms of cell sorting has been facilitated by study of the pattern-forming behavior of homogeneous tissue aggregates placed in contact in organ culture. Typically, the tissue of one of the aggregates of the pair spreads over the surface of the partner aggregate, often enveloping it completely, during the succeeding 2 to 3 d of culture[60,65-68] (Figure 2). Of particular interest to the understanding of cell sorting is the observation that, with only a few documented exceptions, the final arrangement of tissues established by tissue spreading of apposed homogeneous tissue aggregates is the same as that generated by cell sorting of mixed cell aggregates comprised of the same two cell types[60,68,69] (Figure 3). Thus, we have another element that any comprehensive theory of cell sorting must explain: cohering cell populations generate the same patterned arrays from very dissimilar initial organizations; by sorting of mixed aggregates or by the spreading of one tissue over a second when homogeneous tissue aggregates are maintained in apposition. The reported exceptions to this generalization are interesting and are considered later. Finally, it should be noted that the tissue spreading experiment allows the investigator to employ tissue fragments dissected directly from the organism, without the necessity for tissue dis-

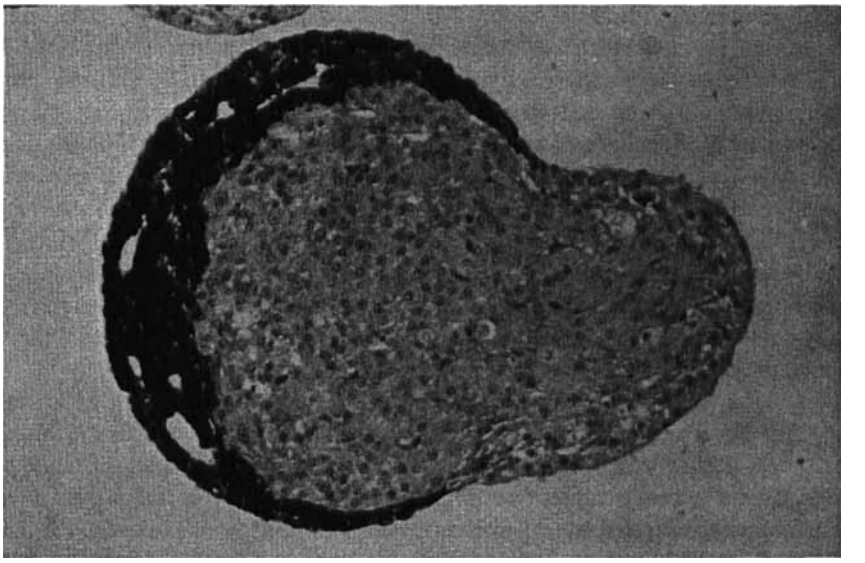


FIGURE 2. Spreading of 10-d-old chick embryo pigmented retinal tissue over the surface of an aggregate of 10-d-old heart tissue. Approximately spherical aggregates of the two tissues were placed in contact in hanging drop culture until they were firmly in adhesion, after which the composite aggregate was maintained in organ culture for 2 d. During this time, the pigmented retinal tissue spread as a monolayer over the surface of the heart aggregate. During envelopment of the heart aggregate, the pigmented retinal tissue reduces the area available for homologous contact while expanding the area of contact with the heterologous tissue.

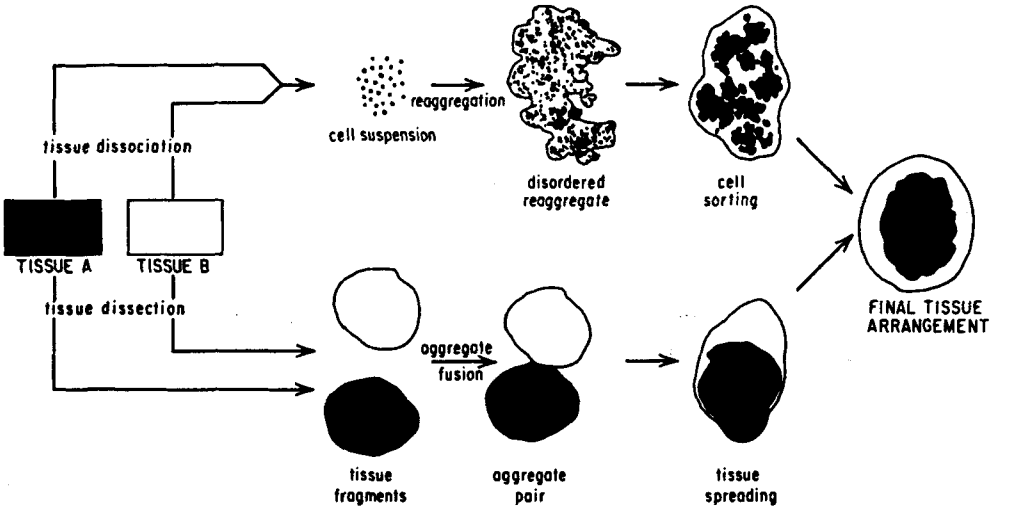


FIGURE 3. Configurational equilibrium. For most binary combinations of tissues, an identical final arrangement of the two tissues results from the sorting out of disordered aggregates prepared by allowing a mixed suspension of dissociated cells to reaggregate (upper half of figure) or by the tissue spreading that follows apposition in the organ culture of homogeneous aggregates of the same two tissues (lower half). Most frequently, the final arrangement is one in which one tissue completely envelopes the second. This behavior is consistent with the differential adhesion hypothesis (DAH), which proposes that the final arrangement of tissues is governed by the relative cohesive strengths and is, thus, independent of the initial organization of the binary aggregate.

sociation and the attendant possibilities for modification of the adhesive character of the cell surface.[70]

III. CELLULAR BASIS FOR CELL SORTING OUT

A. Cell Movement vs. Redifferentiation

Two general kinds of processes can be imagined to contribute to the establishment of homogeneous tissue domains during sorting out: (1) the repositioning of differentiated cells within the confines of the cell aggregate to increase the extent of contact between homologous cells at the expense of heterologous contact, and (2) the redifferentiation of cells that lie outside of their "proper" domain into the cell type characteristic of the position in the aggregate in which they find themselves. The second mechanism almost certainly contributes to pattern formation when the initial aggregate contains relatively undifferentiated cells that are capable during prolonged culture of displaying position-dependent differentiation.[71-73] Most situations of cell sorting, however, appear to involve the rearrangement of stably differentiated cells. A variety of cell markers have been employed to demonstrate this, including endogenous pigmentation differences that allow the investigator to follow cells during the course of sorting,[6,57,74-78] vital staining of cells of one tissue type,[69] labeling with [^{3}H] thymidine,[79-82] and construction of aggregates in which the different tissues are taken from different species with histologically differentiable cell nuclei.[83-88] The present review confines itself to situations in which cell sorting is the consequence of cell rearrangement within the aggregate and does not deal further with cases of cellular redifferentiation.

Two mechanisms of cell movement have been considered for sorting: active, pseudopod-generated locomotion and associative movement. The latter process envisions cell movement to be the consequence of a "zippering up" of homotypic contacts and a resultant reduction in the area of contact between unlike cells.[89] The process does not require the activities of pseudopods as being responsible for cell locomotion. Attempts to distinguish between these possibilities have employed the drug cytochalasin B at concentrations capable of blocking pseudopod-dependent cell locomotion. Although cytochalasin B does block cell sorting in certain tissue combinations,[88,90-94] surprisingly, sorting out proceeds at high concentrations of the drug in certain other tissue combinations.[88,90,92,95] These latter observations indicate that a detectable measure of cell sorting can occur under conditions where active locomotion is inoperative and are consistent with the possibility that associative movement contributes to the reorganization of cells during sorting.

B. The Differential Adhesion Hypothesis

That reproducibly patterned tissue arrays are generated by spontaneous reorganization of disordered heterotypic cell aggregates indicates that properties of the constituent cells are responsible for important features of tissue organization. One of the goals of research in this area has been to identify the relevant cellular properties and the rules by which they operate to determine tissue reorganization during cell sorting and, by implication, tissue organization *in vivo*. The most detailed and most successful explanatory scheme has been the differential adhesion hypothesis (DAH). The DAH proposes that (1) the cells of a given cell type have as one of their phenotypic characteristics a characteristic strength of cohesion to others of their kind, (2) the cells comprising an aggregate are motile, and (3) the final organization of cells is that which maximizes the strength of adhesive interaction summed over all of the adhesive contacts in the aggregate. In this explanation, cell adhesion is defined as the reversible work of adhesion (the work done when one unit of cell surface area is moved from the surface of the aggregate into the interior). The reversible work of adhesion is directly proportional to the negative of the specific interfacial free energy (the change in free energy when the surface area of a spherical cell aggregate is increased reversibly by one unit at the expense of one unit of area of cell-cell contact).

An understanding of how the DAH proposes to explain cell sorting is most easily achieved by the consideration of specific examples. The simplest is an aggregate consisting of only a single cell type that is adhesive to other cells over the entire cell surface. The most stable state for such an aggregate is that which maximizes the area of cell-cell contact and minimizes the area of cell-culture medium contact, *viz.*, a sphere. The DAH proposes that the organization of tissues in aggregates comprised of two or more tissues is governed by the relative values of the works of adhesion for the different possible contacts between cells. In the general case of binary aggregates comprised of A and B cells that are adhesive over their entire surfaces, these will be designated as W_a for the work of cohesion of A cells, W_b for the work of cohesion of B cells, and W_{ab} for the work of adhesion of A cells to B cells. First, consideration can be given to the conditions under which sorting out would be expected, e.g., when the most stable state is one in which homogeneous tissue domains are established. The other possibility is cellular intermingling. In response to the condition of maximization of the total work of adhesion, sorting out is expected when the work of adhesion between unlike cells, W_{ab}, is less than the average of the two works of cohesion, $1/2(W_a + W_b)$. The final, stable organization is expected to be that of cellular intermingling in situations in which the inequality is reversed, $W_{ab} > 1/2(W_a + W_b)$. In the latter situation, the aggregate achieves configurational stability by maximizing the area of heterotypic cell contact. In situations in which cell sorting is expected (when $W_{ab} < 1/2[W_a + W_b]$), the DAH proposes that the final arrangement of tissues is governed by the relative values of the cohesive and adhesive interactions. Complete isolation of the two tissues is expected if the dissimilar cells are unable to adhere to each other (if $W_{ab} = 0$). If $W_{ab} > 0$, the two tissues are expected to remain in contact at the completion of sorting, with the more cohesive tissue arranged in an interior position, allowing it to adopt the spherical shape that maximizes the area of cell-cell contact, and with the less cohesive tissue at the surface, surrounding the interior tissue partially (if the W_{ab} term is less than either W_a or W_b) or completely (if the W_{ab} term is greater than the W of the less cohesive of the two tissues).

C. Other Explanations

A number of alternative explanations have been offered to account for cell sorting. These include suggestions that sorting is a response to chemotactic gradients established in the aggregate,[69,96,97] that differences in the timing of the reacquisition of adhesiveness following dissociation is responsible for sorting,[98-100] the interaction-modulation hypothesis of Curtis,[101] the differential contractility hypothesis,[102] and the specific adhesion hypothesis (SAH).[32] Of these, I consider here only two, the differential contractility hypothesis and the SAH (for reviews, see References 63, 103, and 104). The first proposes that sorting itself and the relative positioning of the sorted out tissues are produced by active cellular contractility induced by exposure to the culture medium, with different cell types showing differing powers of contractility. It is proposed that the more contractile tissue of any given binary combination should segregate to the interior.[102] The SAH proposes that intercellular adhesion evinces a significant degree of tissue specificity with homologous adhesions being stronger than heterologous adhesions. The hypothesis envisions sorting out as occurring as the non-specific adhesive bonds operative in the initial mixed reaggregate are superceded by the specific adhesive interactions that become expressed as the cells repair the damage to their surfaces engendered by tissue dissociation. The specificity of cellular adhesion is thought to involve tissue-specific cell surface adhesive macromolecules.[105-107] In the succeeding sections, the relevant experimental evidence is reviewed as it relates to these proposed explanations for cell sorting, and the implications for an understanding of tissue organization *in vivo* are discussed.

IV. EXPERIMENTAL EVALUATION OF COMPETING HYPOTHESES

The principal contending explanations for cell sorting are the DAH and the SAH. There is little evidence relating to the differential contractility hypothesis, but the observation that an appreciable degree of cell sorting can occur with certain tissue combinations in the presence of concentrations of cytochalasin B adequate to impair actin-based cellular contractility[90,92,95] suggests that the proposed mechanism is not universally applicable. As is developed below, the behavior of cells within heterotypic aggregates is consistent with the DAH, but cells are capable of displaying adhesive specificity when the kinetics of the initiation of adhesion are assayed.

The situation in which the DAH and the SAH conflict is when sorting out results in aggregates with one tissue surrounded completely by the second since it is in this case that the DAH predicts that the heterotypic adhesion, W_{ab}, is stronger than the homotypic adhesions between the cells of the outer tissue layer. This prediction violates the notion of the SAH that cell adhesion displays a strong degree of tissue specificity. When sorting out generates aggregates in which the two tissues show partial or complete isolation, the two hypotheses are not in disagreement. Complete envelopment, however, is the most commonly reported pattern of sorting.

A. Hierarchy

The DAH predicts that quantitative differences in strengths of adhesion are responsible for sorting. This indicates that a simple transitive relationship should exist for ensembles of different cell types that sort out when combined within aggregates. Steinberg[60] has reported just such a hierarchy of sorting out in which a particular tissue in the list below sorts internally to all tissues to its right and externally to all tissues to its left: basal layer of the epidermis (7- to 8-d chick embryo), limb chondroblasts (4-d chick embryo), pigmented retinal epithelium (5-d chick embryo), heart ventricle myocardium (5-d chick embryo), spinal cord (36-h chick embryo), liver (5-d chick embryo), and neural retina (7-d chick embryo). Demonstration of such a transitive relationship in tissue positioning is consistent with the DAH and also emphasizes an important strength of the DAH relative to the SAH: while the SAH can account for sorting out (i.e., the establishment of homogeneous tissue domains), only the DAH can explain both sorting out and the relative placement of the sorted out tissues within the aggregate.

B. Positional Stability

In early studies on cell sorting, Holtfreter demonstrated that the same patterned array of tissues is generated by the sorting out of mixed cell aggregates and by the spreading of one tissue over a partner aggregate when two dissimilar homogeneous tissue aggregates are cultured in contact.[67,69] With some interesting exceptions (to be considered later), the generality of this observation has been well established: the identical arrangement of tissues is established both by cell sorting of mixed aggregates and by tissue spreading of fused homogeneous cell aggregates (Figure 3). This behavior is exactly what is expected if the DAH is valid since this hypothesis claims that the final pattern is determined by relative values of adhesivity and should be independent of the initial arrangement of the tissues as long as the tissues are in contact. The spontaneous spreading of one aggregate over the partner aggregate in the fused aggregate situation is not what would be expected if cell adhesion exhibited a strong measure of tissue specificity because, during spreading, the superficial tissue spontaneously enlarges its area of contact with a heterologous tissue at the expense of the area of homologous cell contact (as spreading occurs, the superficial tissue aggregate deforms from the shape that maximizes homologous contact, the sphere, into a flattened shape that enlarges contact with the partner aggregate) (Figure 2). This aspect of cell behavior is difficult to rationalize, except on the basis of the DAH.

C. Measurement of the Relative Values of the Specific Interfacial Free Energies

As noted earlier, the most stable configuration of an aggregate consisting of a single cell type that is uniformly adhesive over the entire cell surface is a sphere. This maximizes the area of cell-cell contact and minimizes the area of cell-culture medium contact. Work must be done on such an aggregate to deform it from the spherical shape. The relative values of the interfacial free energies for various tissues whose sorting behaviors are known have been estimated by the relative reversible deformability of aggregates using centrifugation[108-110] and compression with a calibrated beam arm.[111,112] Using both techniques, it has been demonstrated that the final extent of deformation resulting from a given applied force is an equilibrium shape because initially spherical aggregates will flatten to the same profile as initially more flattened aggregates will round up when subjected to the same force.[108,112] The time course of the flattening of an initially spherical aggregate involves an initial elastic deformation (the individual cells flatten) that relaxes over several hours, during which time the cells regain their original isodiametric shapes.[110] At this stage in the process, the increased surface area of the deformed aggregate has to have come from a cell surface area that previously was internal in the aggregate and potentially involved in cell-cell or cell-ECM contact.[113] When the relative degrees of deformabilities of various tissues, as estimates of the relative tissue interfacial free energies, were compared to the sorting behaviors of those tissues in binary pairings, the relations predicted by the DAH were observed: the internal position in the sorted out aggregate correlated with a higher value for the interfacial free energy (e.g., reduced ease of deformation). To date, the number of homotypic tissue aggregates that have been analyzed in this manner is small, but the results are consistent with predictions of the DAH.

D. Cell Junctions

Cell-cell adhesion is mediated in part by specialized cell junctions. Included in a list of such specializations are the *adherens* junction, the desmosome, the gap junction, and the tight or *occludens* junction. If cell sorting is the consequence of highly specific adhesive interactions, this should be reflected in an inability of dissimilar cells to establish specialized junctional attachments. This clearly is not so for the *adherens* junction,[74,75] the desmosome,[88,92,95,114,115] and the gap junction.[116,117] When the pattern of sorting of chick embryo cornea and skin was compared to the abundance of desmosomes, it was determined that the interior-segregating tissue (the tissue presumed by the DAH to be the more cohesive) had the higher abundance of desmosomes,[88,118] suggesting that in this situation the density of desmosomes may be the principal factor determining relative cohesivity and sorting behavior.

E. Involvement of the CAMs

The CAMs are integral membrane proteins that function in the adhesion of a variety of cell types. To date, the three best characterized CAMs of the chick embryo are N-CAM, L-CAM, and Ng-CAM. Homologous adhesion proteins have been identified in other vertebrates (for reviews, see References 114 and 119). Organ-cultured aggregates of chick embryo neural retina are capable of reestablishing a recognizable facsimile of the normal layered organization in organ culture, presumably by sorting out of the constituent cell types.[120] F_{ab} fragments of monospecific anti-N-CAM antibodies have been shown to block reestablishment of normal pattern in this system, suggesting that N-CAM plays an important role in sorting and by implication in the normal development of the layered organization of the retina.[120,121] Since N-CAM is an adhesive protein present on a variety of cell types,[122,123] it can be suggested that sorting in this system does not rely on an adhesive molecule of pronounced tissue specificity.

F. Role of the Extracellular Matrix

Two extreme models can be envisioned to describe the character of adhesive interactions in tissues. In one, the individual cells are thought of as being embedded in a voluminous

extracellular matrix without direct cell-cell contact. In this model, the mechanical properties of the tissue would be governed largely by the properties of the matrix. In the other, cell adhesion is viewed as being established directly between the surfaces of adjacent cells, without intervening layers of extraneous material. Although the adhesion of the cells of real tissues usually is effected by a combination of both processes, direct cell contact is the predominating element of the adhesion of epithelial cells, whereas the extracellular matrix plays an important role in the properties of mesenchymal tissues. If specialized cell junctions and the CAMs are involved in the adhesive interactions responsible for the sorting of epithelial tissues, what role is played by the extracellular matrix in the sorting of mesenchymal tissues?

The most thoroughly analyzed situation in which adhesive components of the extracellular matrix have been implicated in a directive role in cell sorting is the sorting of chick embryo heart tissues.[79,124-126] The two most abundant cells in the heart are the mesenchymal cell and the myocardial cell. Myocardium constitutes the wall of ventricles and atria, and mesenchyme is present at the surface (the epicardial mesenchyme) and in the connective tissue of the atrioventricular valves and the outflow tract (the endocardial cushion mesenchyme). Under standard conditions of culture (Dulbecco-modified Eagle's medium + 10% chickem serum), mixed aggregates sort out with the mesenchymal tissue occupying the surface and the myocardium the interior. Sorting in this system appears to be dependent on the deposition of an extracellular matrix containing the matrix adhesion protein, fibronectin. Fibronectin is secreted into the matrix by the mesenchyme, but not the myocardium.[79] Deposition in the matrix is dependent on an as yet unidentified factor in the serum: heart mesenchyme cultured in the absence of serum deposits markedly reduced amounts of fibronectin.[79] In aggregates that have completed sorting, immunocytochemically detectable fibronectin co-localizes with the mesenchymal tissue. Mixed aggregates cultured in a serum-free medium are compact with satisfactory tissue cohesiveness, but they lack fibronectin and fail to sort out. Sorting capabilities are restored if a dispersed preparation of the extracellular matrix is added to the serum-free culture medium. Matrix preparations capable of eliciting sorting have been prepared by extraction of heart fibroblast monolayers with 1 *M* urea (the procedure of Yamada et al.[127] for the preparation of cellular fibronectin) and by hypotonic lysis of confluent heart fibroblast monolayers (the residuum of this contains the insoluble components of the extracellular matrix). The sorted aggregates contain large quantities of fibronectin, which colocalize with the mesenchyme. The ability to reconstitute sorting capabilities with preparations of the extracellular matrix demonstrates an important role in sorting, with fibronectin as the most likely candidate for the specific molecule responsible for sorting.

These observations are interesting in relation to the debate over the involvement of tissue-specific adhesive molecules in cell sorting because fibronectin, with its ability to mediate the attachment of a variety of epithelial and mesenchymal cell types to structural elements of the extracellular matrix, is an exemplar of the highly promiscuous adhesive protein.[128] One way in which fibronectin might mediate sorting in this system would be for the mesenchyme to have a higher adhesive affinity than the myocardial tissue for the fibronectin matrix. This would be expected to allow the mesenchyme to monopolize the fibronectin matrix, excluding the myocytes to fibronectin-depauperate regions of the aggregate. Supporting this notion is the observation that fibronectin-derivatized latex spheres attach in significantly larger numbers to the surfaces of cardiac mesenchyme aggregates than to the surfaces of myocardial aggregates. The effect is specific for fibronectin: bovine serum albumin-coated spheres attach only in very low numbers to either class of aggregate. In summary, these observations provide support at the biochemical level for the suggestion that sorting of heart tissue is a response to a differential affinity of myocardium and cardiac mesenchyme for the adhesive protein fibronectin, a suggestion consistent with the DAH.

G. Position Reversal

One of the most important bodies of evidence favoring the DAH is the stable character of the final arrangement of tissues in heterotypic aggregates; the observation that an identical

final organization of tissues is achieved both by cell sorting of mixed aggregates and by tissue spreading when homogeneous aggregates are cultured in contact (Figure 3). Although, as mentioned previously, most tissue combinations conform to this, a few exceptions have been described.[79,81,129] The sorting of cardiac mesenchyme and myocardium is at present the most thoroughly studied example. Sorting out of mixed aggregates results in the superficial placement of the mesenchymal tissue, whereas myocardium spreads over the surface of mesenchymal aggregates when the two tissues are paired as apposed tissue aggregates. In the one situation, the mesenchymal tissue occupies the surface, and in the other, the mesenchyme occupies the interior. As developed in the previous section, sorting of heart tissues appears to be the result of the deposition of fibronectin in the extracellular spaces by the mesenchyme. We have suggested that sorting of heart aggregates involves the following factors: (1) in culture, the mesenchyme — but not the myocardium — deposits a fibronectin matrix; (2) the initial mixed aggregate lacks fibronectin, presumably as a result of the treatment of the mesenchymal cells with proteases during cell dissociation prior to preparation of the initial mixed aggregate; (3) the eventual establishment of a fibronectin-rich matrix renders the mesenchymal tissue more cohesive than the myocardium; (4) deposition of fibronectin in the mesenchymal matrix is dependent on exposure to a factor in the serum fraction of the culture medium; and (5) this serum factor penetrates the aggregate to a shallow depth, restricting fibronectin deposition to the superficial layers of mesenchyme.[79] Evidence supporting each separate element listed here has been reported previously.[79] Based on this hypothesis, it is possible to account for tissue position reversal. In aggregate-pairing studies, the mesenchymal aggregate has at the time of pairing been maintained in culture for a time adequate to establish a fibronectin-rich matrix and is, as a consequence, more cohesive than the myocardial aggregate. In this situation, the more cohesive mesenchymal aggregate is enveloped by the less cohesive myocardial tissue. In situations of sorting out, the mesenchymal cells in the initial aggregate lack appreciable quantities of associated fibronectin, and tissue segregation occurs concomitant with the establishment of a fibronectin-containing matrix, selectively by those mesenchymal cells that find themselves close to the surface of the mixed reaggregate. According to this explanation, sorting continues as mesenchymal cells wandering from the fibronectin-depauperate interior of the aggregate encounter the fibronectin matrix close to the surface of the aggregate, adhere strongly, and also experience adequate quantities of the stimulatory factor to begin secreting fibronectin themselves. On the basis of this explanation, it is possible to reconcile this situation of tissue position reversal with the DAH.[79]

H. The Kinetics of Cell Attachment: Evidence for Adhesive Specificity

The most convincing evidence for the existence of tissue-specific adhesive interactions has come from the study of the relative rates of initiation of homotypic and heterotypic adhesions. Several procedures have been developed toward this end. Assays include determination of the rates of attachment of (1) freshly-dissociated radiolabeled cells to day-old cell aggregates in stirred suspension culture,[130-135] (2) freshly-dissociated radiolabeled cells to confluent cell monolayers,[136,137] (3) contacting pairs of day-old cell aggregates,[138] and (4) day-old aggregates to cell monolayers.[139-141] With few exceptions, stable homologous adhesions were established more rapidly than heterologous adhesions, even in tissue pairs in which the DAH predicts from the sorting behavior that the heterologous attachment should be the stronger. Although the applicability of these observations to the question of an involvement of tissue-specific adhesive interactions in cell sorting is uncertain (the adhesive interaction presumed by the DAH to be involved in cell sorting can be determined in principal only by reversible measuring procedures and cannot be derived from kinetic measurements),[138,142] the broad agreement of studies from several laboratories, employing a variety of assays and studying a number of different tissue cell types, indicates that adhesive

specificity is frequently displayed in situations that require cells or tissues to initiate adhesion. Clearly the phenomenon is real.

I. Summary

One of the major questions regarding the cellular basis for sorting out is the role played by tissue-specific adhesive interactions. Excellent evidence exists for an involvement of specific adhesive cell recognition in a variety of cellular interactions, including fertilization,[143-146] lymphocyte homing,[147-151] and phagocytic recognition.[152] As discussed previously, a significant body of evidence suggests that the cells of coherent tissues can, under the appropriate assay conditions, express specific adhesive recognition. Analysis of the available evidence, however, indicates that tissue-specific adhesive interactions are not the dominant factor in instances of sorting that result in complete envelopment of one tissue by the partner tissue. Our ability to reconcile the apparent noninvolvement of tissue-specific adhesive interactions in the most commonly observed type of cell sorting (the situation of complete envelopment) with the abilities of the same cell types to display adhesive specificity when asked to initiate new adhesive contacts will probably depend on an improved understanding of the biochemistry of the two situations. Perhaps the cell surface moieties that mediate specific adhesion become modified during prolonged contact between the dissimilar cells that constitute a mixed cell aggregate in a fashion that blocks their contribution to the adhesive interactions responsible for cell sorting. For example, the possible involvement of glycosyl transferases in adhesion permits the enzymatic modification of their oligosaccharide receptors, with an alteration of adhesive function.[153] Alternatively, if one of the cells in an aggregate is capable of secreting into the extracellular matrix one or another of the broad activity matrix adhesion proteins, such as fibronectin, vitronectin, collagen, or laminin, these might come to dominate the adhesive interactions, submerging any effect of molecular species responsible for adhesive specificity.

V. SIMPLE EPITHELIA

In the previous discussion, it was assumed that the cells involved in sorting are uniformly adhesive over their surfaces or, to the extent that adhesion is dependent on specialized cell junctions, that the cell junctions are uniformly spaced. The variety of organizational states that can be generated by tissues composed of uniformly adhesive cells subject to the organizing principals defined by the DAH is decidedly limited and does not include tubules and other epithelial structures.[154] The plasma membrane of simple epithelial cells is polarized in the sense that the apical membrane differs morphologically, biochemically, and functionally from the basolateral membrane.[155-157] A simple way to expand the DAH theory to include simple epithelial structures is to propose that adhesiveness is restricted to the basolateral surfaces, and the apical surfaces of epithelial cells are nonadhesive. Such cells, thus, deviate from the prior supposition of uniform adhesivity. Since the nonadhesive portions of the plasma membranes of associated cells are not able to adhere either to elements of the extracellular matrix or to other cell surfaces, such cells are expected to self-organize as two-dimensional sheets that bound volumes of fluid, either internally by lining tubules or vesicles or externally by forming a surface epithelial layer bounding the aggregate.

A. Vesicular Forms Established by Epithelial Aggregates

Several cases have been reported in which the aggregates established by pure populations of kidney, pancreatic, and thyroid epithelial cells organize in organ culture into vesicular structures in which a single-layered epithelium encloses a volume of fluid.[41,45,158-161] Clearly, these epithelial cell types are capable of restoring the epithelial morphology in the absence of exogenous cues. In other situations, epithelial cells are able to self-organize if cultured

in the presence of an appropriate extracellular matrix. The culture of endothelial cells within a collagen matrix induces the reorganization of aggregates and monolayers into branching networks of linear tubules that resemble capillary plexes *in vivo*. Polarity of the endothelial cells lining the tubules is apparently the correct one with the apical cell surface facing the lumen.[162,163] Endothelial monolayers maintained in fluid medium can form tubules,[164] but the polarity is inverted with the apical surface facing the culture medium.[165]

The polarity of kidney and thyroid epithelial cell types can be identified by morphological markers: the basolateral membrane of both cells is relatively smooth, whereas the apical surface shows closed-packed microvilli and is associated with the juxta-apical junctional complex. In some cases, the final organization of the aggregates is the normal one with the apical poles facing the lumen,[41,59,160] but in others the polarity of the vesicle is influenced by the conditions of the culture, with the apical pole facing the medium in vesicles maintained in liquid medium and the apical pole facing the vesicle lumen in aggregates cultured within gels of native collagen.[47,161,166] Correctly oriented epithelial tubules are also formed when cells of the kidney epithelial cell line (MDCK) are maintained in contact with mesenchyme.[167] Inside-out aggregates spontaneously invert to position the apical pole at the luminal face when the aggregates are transferred from suspension culture to culture within a collagen lattice.[161,166,168-172] Both inside-out and right-side-out epithelial aggregates lack a basal lamina unless the culture medium is supplemented with laminin.[173] Laminin, supplied in the absence of a collagen gel, does not provoke inversion of inside-out aggregates.[170,173]

B. Epithelial Organs

When fragments of simple epithelia of differing adhesiveness of the lateral surfaces are apposed, the same relationships of envelopment described earlier for solid aggregates pertain, albeit in two dimensions. Behavior of this character has been reported for grafts of the epidermis of insects.[266-268] If a sheet of epithelial tissue contains multiple domains of differing adhesiveness (here called blocks to indicate that the domains need not be histologically distinguishable), with the appropriate relative adhesive values for sorting and complete envelopment, the equilibrium arrangement predicted by the DIH would be a bullseye pattern, with the most cohesive block surrounded by annuli comprised of the other blocks in order of progressively decreasing cohesiveness. The DIH further predicts that the overall shape of such an epithelium should be an elongated, hollow cylinder with the most cohesive block at the tip since this shape would minimize the area of contact between successive domains and maximize the area of intra-domain cellular contact. Mittenthal and Mazo[269] have analyzed the shape of arthropod limb segments based on the assumption that the limb epidermis, which produces overall limb shape, shows just this sort of adhesive gradient. In their model, the equilibrium (least energy) shape of a limb segment is the result of minimization of the sum of two terms: the interfacial free energies (to produce an elongated cylindrical epithelial structure) and the mechanical strain resulting from intra-epithelial stiffness, which will resist the bending deformation required to form the tube. A squat tube would result from stiff epithelia with a shallow tip-to-base adhesive gradient; a thin tube from flexible epithelia with a steep adhesive gradient.

C. Tissue Position Reversal

The tissue position reversal exemplified by the lack of identity of the tissue arrangement of myocardial tissue and cardiac mesenchyme following tissue spreading and cell sorting was proposed to result from the unusual conditions for the sorting of heart tissue, *viz.*, the dependence of sorting on the stimulation of fibronectin deposition by a factor in the culture medium that is active only in the superficial cell layers. The inclusion of surface epithelial tissue in cell aggregates can also produce tissue position reversal. In combinations containing the three germ layers from the neurula-stage amphibian embryo, the following tissue po-

sitioning is observed: endoderm surrounds mesoderm, which surrounds subsurface ectoderm,*[112,174] whereas if the aggregates contain, in addition, ectoderm from the surface of the embryo, the following organization of tissues is observed: ectoderm surrounds mesoderm, which surrounds endoderm.[67,69] The rationale for the ability of the superficial layer of neurula-stage ectoderm to produce this change in tissue positioning provided by Steinberg[142] is based on the following propositions: (1) the order of tissue cohesivity (from strongest to weakest) is ectoderm, mesoderm, endoderm; and (2) each cell of the surface layer of ectoderm has a nonadhesive domain that occupies the apical portion of the cell surface. Based on this set of proposals, the DAH predicts both patterns of tissue organization, with the alteration of organization that results from the inclusion of the surface ectoderm being a consequence of the necessity for that tissue to organize as an epithelium that orients the nonadhesive portion of each cell's surface at the surface of the aggregate. The ectoderm is confined to the surface by the presence of the nonadhesive membrane domains on the surface ectodermal cells, and the ordering of the mesoderm and endoderm is dictated by the higher adhesivity of the mesoderm for the ectoderm than the endoderm for the ectoderm.

Several elements of this explanation have been tested experimentally. The relative cohesivity of the three germ layer tissues has been estimated by the degree of compression produced by application of force administered by a calibrated beam arm. Endoderm is the most deformable tissue and subsurface ectoderm the least, consistent with the explanation offered above in the first proposal.[112] While dissociated ectodermal cells attach rapidly to other cells and to artificial surfaces along their basolateral cell surfaces, the apical surface does not adhere to cells or surfaces,[175-178] consistent with the second proposal (above). One unsettled question is why surface ectoderm occupies the surface of mesoderm-ectoderm aggregates, rather than organizing as the innermost tissue with the nonadhesive surface membrane bordering interior cavities, as so many other epithelial tissues do in mixed cell aggregates (Table 1). One possibility is that the basal cell surface is more strongly contractile under the conditions of aggregate culture, so that the apical surface occupies a large fraction of the total surface area of the cell. The most stable packing of epithelial cells with a large fraction of the surface devoted to nonadhesive membrane would be to position this face of the tissue along a convex plane (e.g., to position this tissue at the outer surface of the aggregate). Consistent with this suggestion is the report that isolated pieces of surface ectoderm contract the basal surface to curl the ectoderm into vesicles with the apical surface directed outward.[179]

D. Nonadhesivity of the Apical Surface of Simple Epithelia

The apical surface membrane of simple epithelial cells differs in a number of important respects from the basolateral membrane (for reviews, see References 155 to 157). Of interest to the present subject is the evidence that the apical surface is nonadhesive. The surface ectodermal cells of amphibian embryos,[175-178] chicken embryos,[180] blastocyst-stage mouse embryos,[181] and the surface epithelium of the teleost embryo[182] are apparently completely nonadhesive to cells and foreign particles, whereas the basolateral cell surfaces attach rapidly to cells and particles. Cells introduced into the peritoneum fail to attach to the luminal surface of an intact peritoneal epithelium, but do adhere to sites where the epithelium has been wounded, exposing the lateral cell surfaces and the basal lamina.[183] The luminal surface of normal blood vessels is nonadhesive to blood platelets and other blood cells, whereas platelets and certain other blood cells adhere strongly to areas of damage to the endothelium. A nonadhesive character for the apical surfaces of epithelia maintained in cell culture can be inferred from an inability of particles and dispersed cells to attach to the upper surface of confluent monolayers.[184-187]

* In anurans, the ectoderm is more than one cell thick, with only the surfacemost layer possessing a nonadhesive surface.

The biochemical mechanisms of nonadhesiveness have not received much study. One contributing factor appears to be an absence at the apical surface of the molecular species thought to be responsible for the adhesiveness of the basolateral cell surfaces.[188-190] The nonthrombogenic character (nonadhesivity to blood platelets) of the luminal surface of the endothelium appears to be attributable to electrostatic repulsion between the negatively charged surfaces of the endothelium and the blood platelet.[191,192] The luminal surface of the endothelium bears an unusually high density of sialic acid residues which contributes to its high electronegative surface charge.[193]

E. Other Mechanisms Potentially Responsible for Epithelial Organization

It is suggested above that the organization of epithelial cells as a two-dimensional cell sheet is a consequence of the presence of a patch of nonadhesive membrane on each cell. At configurational equilibrium, the argument holds that these patches must face on fluid-filled spaces since they are unable to participate in cell-cell or cell-matrix attachment. The differences in organization between simple and stratified epithelia are proposed to result from differences in the distribution of adhesive elements such as desmosomes: simple epithelia are adhesive only at the basolateral surfaces, whereas the cells of the intermediate layers of stratified epithelia are adhesive over the entire cell surface.[114,194] Other processes can be imagined which might substitute for or reinforce the operation of the nonadhesive membrane patches for the structuring of simple epithelia. Goel and Leith[154] suggest, on the basis of mathematical modeling studies, that quantitative differences in adhesive affinities of different regions of the cell surface can confer epithelium-forming capabilities without the necessity for nonadhesive domains. Presumably, the juxta-apical junctional complex[195] could serve as a localized region of strong adhesiveness that would stabilize the structure of epithelia in this manner. A strong adhesive affinity for a planar substrate could also contribute to the organization of cells in monolayered sheets.[196,197] An obvious example of this is the monolayered organization of cells cultured on adhesive glass or plastic surfaces.[198] Even mesenchymal cells that *in situ* are organized in three dimensions organize as monolayers in cell culture. The basal lamina might serve the role of planar adhesive surface for the organization of epithelia as two-dimensional sheets.[199,200] It should be noted that the presence of a basal lamina is not necessary for maintenance of epithelial organization: epithelial follicles maintained in organ culture retain an epithelial organization without the presence of a basal lamina.[59,159,170,172,173,201-203] Finally, the vectorial transport of solute into intercellular spaces sealed by *zonula occludens* cell junctions may serve to dilate the spaces and organize the tissue lining the spaces as an epithelium.[160,203,204] Although this process appears to influence the volumes of closed epithelial bodies *in vitro*,[203] it must be remembered that most epithelia in the body line spaces accessible to the outside (the lumens of the digestive, respiratory, excretory, and reproductive systems and those of the epidermal ducts all communicate with the outside), providing a ready escape for accumulated fluids. In summary, several processes, mostly adhesive in nature, potentially contribute to the organization of simple epithelia as monolayers. An unsolved problem is assessment of the relative contributions of each of these processes to the form of specific epithelia. Investigation of the ability of aggregated populations of epithelial cells in culture to reconstruct epithelial sheets will continue to be an important system in this analysis.

VI. ADHESIVE DIFFERENTIALS AND ANATOMICAL STRUCTURE

A useful feature of the cell sorting system is the opportunity that it affords for experimental intervention. This system allows a program of analysis that might appropriately be designated experimental histology. The principal goal of these studies is to discover the basic principle governing the anatomical relationships of tissues by studying the effects of various pertur-

bations on the pattern-forming abilities of cultured aggregates. The investigator can manipulate the cell types present and the initial spatial arrangement of the cells and, in favorable cases, can study the biochemistry of pattern regulation by manipulating the presence or activities of particular macromolecular constituents of the cell surface and the extracellular matrix. In this section, selected examples are described in which either the experimental methodology or the DAH have provided important insights into normal morphology.

A. Morphogenetic Movement

Directed cell movement is an important process contributing to the development of histological organization during embryogenesis. Individual cells, cell groups, and entire tissues move in the embryo in a series of precisely orchestrated morphogenetic movements. Cells in particular sites in the embryo begin moving at precisely defined stages of development, move over well-defined paths, and ultimately localize in specific association with other tissues, often at sites quite distant from their original locations in the body (for reviews, see References 1 to 4). For all of the variability of the details of cell behavior across the spectrum of different morphogenetic movements, the sorting out of predetermined cells from a random array into the definitive organization has not, to this author's knowledge, been reported to occur. Several morphogenetic movements feature the migration of individual cells in a fashion that completely scrambles original nearest-neighbor relationships (examples include the migration of trunk neural crest[2] and the cells of the blastula stage teleost embryo[205-208]), but in these cases, the cells are undetermined at the initiation of migration, and cell determination is regulated by the environments through which the cells migrate and in which the cells finally localize.[205,209] Sorting of predetermined cell types is not involved in morphogenesis *in vivo*. Cell sorting is of interest to the developmental biologist, not because it faithfully mimics the details of particular normal morphogenetic events, but because it provides an opportunity to explore the way in which tissue cohesion regulates morphogenetic performance. In this section, lessons are applied which were learned from the study of cell sorting to two categories of morphogenetic migratory events: tissue spreading and haptotactic migration.

In certain cases, embryonic tissue movements occur as the spreading of a sheet of one tissue either over an extracellular matrix such as the basal lamina or, in some cases, directly over the surface of a second tissue. Examples include the migration of lateral mesoderm between ectoderm and endoderm in vertebrates,[210-212] the epibolic spread of the embryo itself over the yolk mass in birds[213-216] and teleosts,[217-219] the spreading of the epicardium over the myocardial surface of the heart,[220,221] and epithelial migration during epidermal wound closure.[222,223] As has been developed in preceding sections, the DAH proposes to account for cell sorting and tissue spreading in organ-cultured cell aggregates by the same set of general principles. The DAH proposes that tissue spreading can be explained by the operation of the simple principle that progressive spreading will occur when the tissue cohesiveness is less than the adhesiveness of that tissue to the substratum. This proposition is inapplicable to situations in which spreading is produced by the passive towing of the epithelial sheet by a second tissue (e.g., teleost epiboly[219]), but remains a viable possibility for situations involving active migration of the epithelial sheet.

Migrating cells establish temporary adhesive contacts with the substratum to provide traction for movement.[224,225] Haptotaxis is the situation in which local differences in the adhesive character of the substratum impart direction to locomotion, with cells migrating preferentially from locations at which they adhere relatively poorly to positions that afford stronger adhesion. Haptotactic migration has been shown to be capable of directing the locomotion of a variety of cells in monolayer cell culture, including nerve axons,[226,227] fibroblasts,[228,229] neutrophil leukocytes,[230] and tumor cells.[231,232] Gustafson and Wolpert[233] suggested that haptotactic migration is a consequence of competition between pseudopods:

presumably those pseudopods contacting a more adhesive area of the substratum will be less readily distracted than those pseudopods adherent to less adhesive regions. As pseudopods contract, the adhesion sites that give way will be the weaker ones, and the cell will gravitate toward the contact sites associated with the more adhesive regions of the substratum. They have suggested that haptotactic migration regulates the locomotory behavior *in vivo* of the mesenchyme in the gastrula-stage sea urchin embryo.[233] Haptotaxis appears also to be responsible for directing the cellular migration necessary for elongation of the pronephric duct rudiment in the salamander embryo.[234] Elongation of the duct primordium is produced by a caudad-directed migration of locomotory cells at its tip. Tip cells migrate over the surface of the lateral mesoderm at the ventrolateral border of the somites. It is proposed that the duct cells are guided in their migration by a craniocaudally traveling adhesive gradient in the mesodermal substratum. The preferential migration of certain classes of neurites along glial cells during development of the laminated organization of the mammalian cerebrum and cerebellum appears to represent a situation of haptotaxis, with the preferential adhesion of neurite for glial cell[226] ensuring that the radial glial cell surface serves as the pathway of choice for cell migration.[235] The integral membrane adhesive molecule Ng-CAM (L1 in mammals) appears to participate in the adhesive interactions of neurite and glial cell during these migrations.[236,237] Clearly, haptotaxis and differential adhesiveness are closely related ideas.

B. Segregation of Organ Primordia

An important morphogenetic process is the progressive segregation or compartmentalization of the embryo into discrete parts. This can be considered as occurring at three hierarchical levels: (1) early segregation of the germ layers and their compartmentalization into major regions, (2) later segregation of organ primordia from larger and previously homogeneous tissues, and (3) the segregation of specific tissues from the formerly homogeneous tissue of an organ rudiment. The first class of events is exemplified by the segregation of hypoblast and epiblast in mammalian and avian embryos, segregation of mesoderm into notochord, somitogenic plate, intermediate mesoderm and lateral mesoderm, and the subdivision of the ectoderm into neural plate and epidermal ectoderm. Numerous examples of the segregation of organ primordia can be cited. The limb mesoderm segregates from the adjacent flank mesoderm; the rudiments of the pronephros, mesonephros, and metanephros segregate from the tissue of the intermediate mesoderm; the primordia of the gonads segregate from the mesonephrogenic rudiments; the individual somites segregate from the somitogenic plate mesoderm; localized domains called placodes segregate from ectoderm as early events in the differentiation of nasal epithelium, lens, auditory epithelium, cranial ganglia, and hair, scale, and feather papillae; and the gut tube segregates into the several organs of the digestive and respiratory systems. Subsequent development of particular organs frequently involves segregation of specific tissues and compartmentalization of the rudiment into several distinct parts. For example, limb-bud somatic mesoderm segregates an internal core of chondrogenic tissue from a superficial layer of mesenchyme, the secretory tubules of the mesonephros and metanephros segregate from the nephrogenic mesenchyme, and a variety of distinct cell types segregate into specific domains of the developing central nervous system. Useful reviews of the details of these events are found in Carlson[238] and Hopper and Hart.[239] In as diverse a list as this, it is certain that a variety of different processes contribute to the segregation processes. Inductive influences play a central role in the initiation of local programs of cell differentiation.[240] The folding of epithelia, differential growth, and localized cell death also participate. One potentially important mechanism appropriate to the present review is the acquisition of adhesive characteristics of the tissue of the prospective rudiment that differ from those of the larger tissue mass of which it is a part. Once such adhesive differences are established, operation of the factors that allow cell sorting *in vitro* would

presumably operate *in vivo* to delineate the boundaries of the organ rudiment from adjacent tissue and to initiate the process of segregation. In this case, segregation occurs without actual cell movement; the change in adhesive properties is thought of as the event that precipitates morphogenetic delimitation of the organ rudiment or specific tissue. The alteration of adhesive affinities could involve changes in the strengths of cell-cell adhesiveness or could involve localized changes in the composition of the extracellular matrix.

Evidence for the operation of adhesive differentials at all three levels can be cited. The observation that chick embryonic epiblast and hypoblast sort out in organ-cultured aggregates is consistent with the hypothesis that the development of adhesive differentials contributes to their segregation *in vivo*.[241] Heintzelman et al.[111] demonstrated an alteration of tissue spreading behavior of early limb-bud mesoderm in organ culture in combinations with flank somatic mesoderm, consistent with the suggestion that an increase in tissue cohesiveness accompanies the delimitation of the limb mesoderm from nonlimb flank mesoderm. Observation of the ability of transplanted pronephric duct rudiments to fuse with the host embryo's pronephric duct, without fusing with other regions of mesoderm (lateral plate and somite) to which the pronephric duct nevertheless adheres, has suggested that the delimitation of the pronephric duct from contiguous tissues is a consequence of adhesive differentials of the character that produce cell sorting in experimentally produced mixed aggregates.[234,242-244] Finally, the ability of chondrogenic tissue to sort out from limb-bud mesenchyme suggests an involvement of adhesive differentials in the establishment of the discrete character of these two tissues during limb development.[245,246] The segregation and compartmentalization of the embryo into its parts are central features of morphogenesis. If the arguments developed in this section are correct, then it can be suggested that one of the categories of features that become established during cell differentiation is the adhesive differentials that then act in compartmentalization.

C. Tissue Stability and Intercellular Invasion

One of the most fundamental, and least studied, questions of experimental anatomy is the characterization of the processes that impose organizational stability onto the histotypic relationships of tissues. The temporal stability of tissue structure, although taken for granted by most students of anatomy and histology, is a subject of interest, both because of the functional importance of stability (it is difficult to imagine that the complex and intergrated functions of the organs could be maintained in the face of a progressive loss of spatial organization) and from the realization that the cells of many of the tissues of the adult organism retain the capabilities for active cellular locomotion, as evidenced by the locomotory performances of cells placed in monolayer cell culture[247] and cells engaged in wound repair.[248-252]

The interest of tissue stability as a subject for experimental investigation is further supported by the realization that the state opposite stability is intercellular invasion. Anatomical stability means that the component cells remain within the confines of the parent tissue for the life of the organism. Intercellular invasion is the intrusion of the invasive cells into the fabric of contiguous tissues. During invasion, cells move across tissue boundaries, destabilizing previous anatomical relationships (for reviews, see References 253 to 255).

Several potential mechanisms can be imagined that might serve to restrain the inherent locomotory capabilities of tissue cells, with the result being the stabilization of anatomical structure (Table 2). Intercellular invasion can be considered in relation to dysfunction of these stabilizing processes (Table 3). Experimental investigation involves the determination of the relative contributions of the processes listed in Table 2 to tissue stability and those listed in Table 3 to invasion. Of particular interest to the present review is the operation of differential adhesion. The observation that experimentally intermingled cells of mixed aggregates will sort out into homogeneous tissues indicates that the processes stabilizing tissue

Table 2
PROCESSES CONTRIBUTING TO THE STABILIZATION OF THE POSITIONING OF POTENTIALLY MOTILE CELLS

I. Static processes: the cells of coherent tissues are normally stationary
   1. Contact inhibition of pseudopodial activity: pseudopodial protrusion (and hence cellular locomotion) is absent at areas of cell-cell contact
   2. Reversible loss of the contractile machinery: one or several of the elements essential for locomotion are absent from quiescent tissue cells and need to be synthesized prior to the acquisition of locomotory ability
   3. Cellular rigidity: save for the small portion of the cell potentially involved in pseudopodial activity, the cell is undeformable and hence unable to squeeze through the clefts between neighboring cells
   4. Impenetrable intercellular matrix: all cells of a tissue are invested in a coherent and impenetrable extracellular matrix that prevents any translocation
   5. Strong cell-cell adhesion: the force of locomotion is too feeble to break cell-cell adhesive contacts, so cells are unable to separate from their neighbors; adhesion may be mediated by cell junctions (desmosomes, gap, and tight junctions) or by elements of the intercellular matrix (fibronectin, laminin, collagen)

II. Dynamic processes: the cells of coherent tissues are permitted to move about within the confines of the parent tissue, but never migrate beyond its borders
   1. Mechanical barriers at tissue boundaries: movement across tissue boundaries is prevented by impenetrable mechanical impediments such as the basal lamella, which do not, of themselves, prevent movement of cells within the tissues
   2. Tissue recognition: cells do not move across tissue borders because the relative values of the specific interfacial free energies of the apposed tissues favor sorting into discrete tissues rather than intermingling of dissimilar cell types; this does not constrain cells from moving about within the tissues, just from moving from one tissue into the next

Table 3
PROCESSES CONTRIBUTING TO INVASION

I. Tissue growth: increase in the volume of the invasive tissue causes its intrusion into contiguous tissues

II. Cellular locomotion: active, pseudopod-directed cellular locomotion contributes to interpenetration of invading and host tissues
   1. Absence of contact inhibition of pseudopodial activity: the continued protrusion of pseudopods (and hence continued locomotion) is not suppressed by contact with host tissue cells
   2. Constitutive expression of the locomotory machinery: the contractile machinery and other elements necessary for motility are intact and operational in invasive cells under conditions where essential elements are lacking in noninvasive tissue cells
   3. Cellular deformability: invasive cells are highly deformable and able to migrate through the interstices in the extracellular matrix and narrow spaces between cells of the host tissue
   4. Lytic enzymes: invasive cells display at the cell surface and/or release into the external milieu high levels of lytic enzymes that disrupt the intercellular matrix and the basal lamella and weaken the adhesive interactions between host tissue cells, allowing migration through what would otherwise be an impenetrable tissue
   5. Cell-cell adhesion is weak: the adhesive interactions binding adhesive cells to neighboring cells are inadequate to resist the forces generated during cellular locomotion
   6. Tissue recognition:
      a. The relative specific interfacial free energies of host and invasive tissues favor cellular intermingling
      b. The migration of the invasive cells is unresponsive to interfacial free energy considerations that would, with apposed noninvasive tissues, favor cell sorting
   7. Chemotaxis: migration of invasive cells into a host tissue is elicited by a concentration gradient of a chemoattractive or chemorepellent chemical

III. Host tissue lysis: destruction of contiguous host tissues provides space for expansive growth and/or immigration of the invading tissue

architecture may include the dynamic ability to actively correct defects in organization. Application of the DAH to this situation implies that an important feature in stability is the appropriate relationship of adhesive strengths of the cells of apposed tissues (*viz.*, stability is ensured if W_{ab} is less than $1/2(W_a + W_b)$). This argument for a role for adhesive differentials in histologic stability is closely related to the argument developed in the preceding section that adhesive differentials potentially play a role in the segregation of organ and tissue rudiments during embryonic development.

The ability to manipulate the activities of adhesive proteins in cultured cell aggregates with antibodies and the alteration of the composition of the extracellular matrix offers an opportunity to develop a biochemical understanding of stability on one hand and invasion on the other. The apparent importance of fibronectin for the sorting of heart tissues suggests that this protein may play an important role in the stabilization of mesenchymal tissues *in vivo*. This suggestion is consistent with the suggestion that the invasive character of sarcomas may result from a decreased presence of fibronectin in the extracellular matrix.[256,257]

D. Tissue Stability and Invasion during Heart Development

Study of cell sorting of cardiac mesenchyme-myocardium aggregates has been used to analyze the mechanisms determining the spatial relation of the two principal tissues of the chick embryo heart at a stage when its form is basically that of the adult (e.g., 10 d of development). Large accumulations of mesenchyme are present in two locations: the epicardial mesenchyme at the surface of the heart wall and the endocardial cushion mesenchyme constituting the connective tissue of the atrioventricular valves and the outflow tract. In both loci, myocardium and mesenchyme are in direct apposition without an intervening basal lamina. The organization of the myocardium-mesenchyme interface is very different at these two sites. The two tissues are strictly segregated, with a planar border at the epicardial mesenchyme-myocardium interface, but are intimately intermingled at the endocardial cushion-myocardium interface. The intermingling of cells seen at 10 d of development is apparently brought about by active invasion across the mesenchyme-myocardium interface beginning at about day 6 to 7 of development. Of interest is the possibility that the factors that determine sorting behavior *in vitro* operate *in vivo* to determine the spatial relationship of mesenchyme and myocardium. Specifically, the possibilities exist that the intermingling of myocardium and mesenchyme at the cushion mesenchyme-myocardium interface *in vivo* is analogous to the failure of cell sorting *in vitro* in the absence of matrix fibronectin, and the strict segregation of the two tissues at the epicardial mesenchyme-myocardium border is analogous to the sorting of mesenchyme from myocardium when fibronectin is present in the extracellular matrix. Determination of the distribution of fibronectin in the extracellular matrix of the 10-d-old chick embryo heart is encouraging: the epicardial mesenchyme contains an abundance of fibronectin, whereas the endocardial cushion mesenchyme contains barely detectable amounts.[125] Thus, the quantity of fibronectin in the extracellular matrix of heart mesenchymal tissues may determine whether the interface will be stable or whether invasion across that interface will occur.

E. Positional Information

Position-specific cellular differentiation plays a critical role in the positioning of tissues in the developing organism. In some situations, interactions between inducing and reacting tissues serve as the basis for positional specificity of differentiation.[240] in other situations, accumulations of determinative cytoplasmic factors established before or soon after fertilization determine later events of position-specific differentiation. However, many situations show little evidence for either mechanism for determining local patterns of tissue differentiation. It has been suggested that, in certain selected examples, the spatial control of differentiation is dependent on gradients or other nonuniform patterns of regulatory factors

that determine patterns of differentiation during morphogenesis. The vertebrate limb is an extensively studied example of a system in which morphogenesis depends on regional patterns of differentiation during morphogenesis. The vertebrate limb is an extensively studied example of a system in which morphogenesis depends on regional patterns of differentiation to yield, for example, the proximo-distal sequence of the limb parts, girdle, upper arm, elbow, forearm, wrist, and digits. It has been suffested that one type of information that is employed by limb mesoderm to regulate position-specific morphogenetic differentiation is adhesive in character, with the limb mesoderm showing a proximo-distal gradient in adhesivity, with the most adhesive tissue at the distal end. Evidence for this has emerged from the study of salamander limb regeneration: the regeneration blastemas (e.g., the accumulations of undifferentiated mesoderm at the tip of the regenerating limb) from distal levels are enveloped in organ culture by blastema tissue from more proximal levels.[270] This is reminiscent of the proximo-distal gradient in adhesiveness proposed for the epidermis of the arthropod limb.[269] It has been suggested that these adhesive gradients may contribute to the establishment of an elongated form of the developing limb at stages prior to stabilization by the exoskeleton in the arthropod or the endoskeleton in the vertebrate and, as indicated above, may also contribute to the spatial information assumed to be required for initial pattern formation and for orderly regeneration.[5,271]

F. Retinotectal Innervation

Anatomically, the most complex organ system clearly is the vertebrate nervous system. The high degree of precision of the neuronal connections within the nervous system is essential for its complicated and precise functioning. The pattern of neuronal connections is achieved during development in large measure by precisely orchestrated morphogenetic movements of neuroblasts and nerve-axon growth cones. One of the major challenges facing developmental neurobiology is understanding the mechanisms that direct the migratory movements in the developing nervous system and that regulate the ultimate patterning of synaptic connections. One of the best-studied systems for exploring these problems is the organization of retinotectal connections in lower vertebrates. During development, the axons of the optic nerve migrate to the surface of the optic tectum and establish a precise region-by-region pattern of synaptic connections by which particular groups of tectal cells receive visual input from small, precisely defined regions of the retina.

Research on the establishment of the retinotectal map has sought to explain the factors that guide the axonal tips to the appropriate sites on the tectum. Two competing categories of hypotheses have guided most of the early studies in this field: (1) the notion that the map is established in response to highly specific positional cues, presumably adhesive in nature, that code the surfaces of optic nerve axons and the target tectal cells[258] and (2) the notion that the retinotectal projection is highly plastic, with the ability to respond to surgical intervention by expansion or contraction of the map (for review, see Reference 259). Conflict between the two opposing views has recently been resolved by an explanatory scheme that draws on basic concepts of the DAH.[259] The hypothesis proposes that development of the map is responsive to four categories of cell-cell interaction: (1) a strong, position-independent adhesion between optic nerve axons and between axon terminals and the tectum, (2) a less strong repulsion or competition between optic nerve terminals, (3) a weak position-dependent adhesion between optic nerve axons and between axon terminal and tectum oriented along the dorsoventral axis of the eye, and (4) a weak position-dependent adhesion between optic nerve axons and between axon terminals and tectum oriented along the anteroposterior axis of the eye. The model proposes that the retinotectal map is the array of connections that maximizes the adhesive interactions (interactions 1, 3, and 4 above) and minimizes the competitive interaction (interaction 2 above). This notion of the tissue pattern being determined by a maximization in quantitative terms of the sum total of adhesive and competitive

interactions in the system is a direct adaptation of the scheme of tissue pattern determination postulated by the DAH. The predictions of the model for various experimental manipulations have been determined by a computer determination of the "best-fit" matching of axon termini and tectal position. The model has been remarkably successful, reconciling large bodies of apparently conflicting observations under a single embracing explanatory scheme.

G. Categories of Adhesive Interaction in Development

Probably the most significant contribution of the DAH to our understanding of tissue patterning is the philosophical notion that complex organization at the anatomical level need not always require the operation of highly specific adhesive interactions at the level of the different cell types. Cell-cell and cell-extracellular matrix adhesion are, of course, mediated by particular cell surface molecular species, but it is suggested that some of these may show a fairly broad distribution, being present on several different cell types or being capable of interacting with related surface molecules on other cell types. A given cell type should be able to adhere to any other cell with which it shares a homologous adhesion system, but should fail to adhere to cells with which it shares no homologous systems. An interesting form of this latter specificity is shown by two categories of cell-cell adhesive systems that can be distinguished by their sensitivity to inactivation by proteases and the requirement for Ca^{2+}.[260] A variety of cell types possess both systems.[261-263] However, by controlling the exposure to proteases and the concentration of Ca^{2+} during aggregation, it is possible to produce subpopulations of a given cell type that show only one or the other system. Cells possessing only the Ca^{2+}-independent adhesion system do not adhere to homologous or heterologous cells possessing only the Ca^{2+}-dependent system, whereas even heterologous cells possessing either the one or the other system coaggregate.[261-263]

A major challenge is the determination of the relative importance for the morphogenesis of adhesive interactions specific for particular cell types and adhesive interactions that show only limited specificity. Certainly, the biochemistry of the latter class of adhesive interactions is the more advanced. We are now in possession of a sophisticated understanding of an important family of integral membrane adhesion molecules, the CAMs, and of several matrix adhesion proteins (the collagens, fibronectin, laminin, vitronectin, etc., most of which employ the amino acid sequence Arg-Gly-Asp[272,273] for recognition of cell surface receptors). Individual members of both classes of adhesion molecules contribute to the adhesive interactions of a remarkably broad spectrum of different cell types. The CAM's experience stereotyped patterns of developmental regulation of the two primary CAMs, N-CAM and L-CAM,[123,264,265] corresponding to important events of morphogenetic change and implying a functional role in morphogenesis.[122] N-CAM is an example of a Ca^{2+}-independent adhesion molecule and L-CAM is an example of a Ca^{2+}-dependent molecule.[119] Thus, although tissue-specific adhesive molecules may play important roles in tissue morphogenesis, our current biochemical understanding is strongest for adhesive molecules with only limited specificity of action.

VII. SUMMARY

The question posed by the science of analytical histology is how the properties and interactions of the components of the tissues determine their organization in the organs. The relevant components of the tissues are the cells and the extracellular matrix. The ability of cohering populations of cells to self-assemble structured tissues by cell sorting out offers an important opportunity for the experimental study of the mechanisms by which the cells and extracellular matrix interact to determine structure. The investigator can manipulate the initial organization and the cellular composition of the system and, in favorable situations, the composition of the extracellular matrix and the activities of candidate adhesive molecules.

It can reasonably be expected that the recent progress in the characterization of the molecular species involved in cell-cell and cell-extracellular matrix interaction will allow the analysis of the molecular basis of tissue organization, with study of the self-assembly of tissue structure during sorting out playing an important role in this analysis.

The importance of the differential adhesion hypothesis is its success in describing the rules by which macroscopic tissue structure is governed by the adhesive interactions of cell with cell and cell with extracellular matrix. The DAH describes how the physical forces of cell-cell and cell-matrix adhesion determine structure. Elucidation of the particular adhesive molecules involved in these interactions (e.g., the CAMs, junctional proteins, and matrix adhesion molecules) will yield an explanation at the biochemical level. A complete understanding of structure requires both levels of explanation.

ACKNOWLEDGMENTS

Original research reported in this review was supported by NSF Grant No. 24181.

REFERENCES

1. **Armstrong, P. B.,** The control of cell motility during embryogenesis, *Cancer Metast. Rev.*, 4, 59, 1985.
2. **Erickson, C. A.,** Control of neural crest cell morphogenesis, in *Developmental Biology. A Comprehensive Synthesis*, Browder, L. W., Ed., Plenum Press, New York, 1986, 481.
3. **Thiery, J. P., Duband, J. L., and Tucker, G. C.,** Cell migration in the vertebrate embryo: role of cell adhesion and tissue environment in pattern formation, *Annu. Rev. Cell Biol.*, 1, 91, 1985.
4. **Trinkaus, J. P.,** *Cells Into Organs*, 2nd ed., Prentice-Hall, Englewood Cliffs, N.J., 1984.
5. **French, V., Bryant, P. J., and Bryant, S. V.,** Pattern regulation in epimorphic fields, *Science*, 93, 969, 1976.
6. **Holtfreter, J.,** Experimental studies on the development of the pronephros, *Rev. Can. Biol.*, 3, 220, 1944.
7. **Dan-Sohkawa, M., Yamanaka, H., and Watanabe, K.,** Reconstruction of bipinnaria larvae from dissociated embryonic cells of the starfish, *Asterina pectinifera*, *J. Embryol. Exp. Morphol.*, 94, 47, 1986.
8. **Fehon, R. H. and Schubiger, G.,** Dissociation and sorting out of *Drosophila* imaginal disc cells, *Dev. Biol.*, 108, 465, 1985.
9. **Galtsoff, P. S.,** Regeneration after dissociation (an experimental study on sponges). I. Behavior of dissociated cells of *Microciona porifera* under normal and altered conditions, *J. Exp. Zool.*, 42, 183, 1925.
10. **Garcia-Bellido, A.,** Pattern reconstruction by dissociated imaginal disc cells of *Drosophila melanogaster*, *Dev. Biol.*, 14, 278, 1966.
11. **Gierer, A., Berking, S., Bode, H., David, C. N., Flick, K., Hansmann, G., Schaller, H., and Trenkner, E.,** Regeneration of hydra from reaggregated cells, *Nature (New Biol.)*, 239, 98, 1972.
12. **Giudice, G.,** Restitution of whole larvae from dissociated cells of sea urchin embryos, *Dev. Biol.*, 5, 402, 1962.
13. **Lesseps, R. J.,** Culture of dissociated *Drosophila* embryos; aggregated cells differentiate and sort out, *Science*, 148, 502, 1965.
14. **Wilson, H. V.,** On some phenomena of coalescence and regeneration in sponges, *J. Exp. Zool.*, 5, 245, 1907.
15. **Yamanaka, H., Tanaka-Ohmura, Y., and Dan-Sohkawa, M.,** What do dissociated embryonic cells of the starfish, *Asterina pectinifera*, do to reconstruct bipinnaria larvae?, *J. Embryol. Exp. Morphol.*, 94, 61, 1986.
16. **Garber, B., Moscona, A. A., and Piddington, S.,** Aggregation *in vivo* of dissociated cells. I. Reconstruction of skin in the chorioallantoic membrane from suspension of embryonic chick and mouse skin cells, *J. Exp. Zool.*, 155, 179, 1964.
17. **Garber, B., Kollar, E. J., and Moscona, A. A.,** Aggregation *in vivo* of dissociated cells. III. Effect of state of differentiation of cells on feather development in hybrid aggregates of embryonic mouse and chick skin cells, *J. Exp. Zool.*, 168, 455, 1968.
18. **Moscona, A. A.,** Studies on stability of phenotypic traits in embryonic integumental tissues and cells, in *The Epidermis*, Montagna, W. and Lobitz, W. C., Eds., Academic Press, New York, 1964, 83.

19. **Weiss, P. and James, R.,** Skin metaplasia in vitro induced by brief exposure to vitamin A, *Exp. Cell Res.*, Suppl. 3, 381, 1955.
20. **Weiss, P. and Taylor, A. C.,** Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation, *Proc. Natl. Acad. Sci. U.S.A.*, 46, 1177, 1960.
21. **Orr, M. F.,** Histogenesis of sensory epithelium in reaggregates of dissociated embryonic chick otocysts, *Dev. Biol.*, 17, 39, 1968.
22. **De Long, G. R.,** Histogenesis of fetal mouse isocortex and hippocampus in reaggregating cell cultures, *Dev. Biol.*, 22, 563, 1970.
23. **Ezerman, E. B. and Kromer, L. F.,** Development and neuronal organization of dissociated and reaggregated embryonic cerebellum after intracephalic transplantation to adult rodent recipients, *Dev. Brain Res.*, 23, 287, 1985.
24. **Hemmendinger, L. M., Garber, B. B., Hoffmann, P. C., and Heller, A.,** Selective association of embryonic murine mesencephalic dopamine neurons in vitro, *Brain Res.*, 222, 417, 1981.
25. **Ishii, K.,** Reconstruction of dissociated chick brain cells in rotation-mediated culture, *Cytologia*, 31, 89, 1966.
26. **Levitt, P., Moore, R. Y., and Garber, B. B.,** Selective cell association of catecholamine neurons in brain aggregates in vitro, *Brain Res.*, 111, 311, 1976.
27. **Lindner, J. and Schachner, M.,** Immunochemical localization of cell type-specific markers in reaggregating cell cultures of mouse cerebellum, *Cell Tissue Res.*, 227, 677, 1982.
28. **Peterson, G. R., Fischer, P. H., and Burkhalter, A.,** Rotation cultures from different regions of embryonic chick brain. I. Biochemical and morphological characteristics, *Neurobiology*, 4, 210, 1974.
29. **Levak-Svajger, B. and Moscona, A. A.,** Differentiation in grafts of aggregates of embryonic chick and mouse cells, *Exp. Cell Res.*, 36, 692, 1964.
30. **Moscona, A. and Moscona, H.,** The dissociation and aggregation of cells from organ rudiments of the early chick embryo, *J. Anat.*, 86, 287, 1952.
31. **Moscona, A.,** Development of heterotypic combinations of dissociated embryonic chick cells, *Proc. Soc. Exp. Biol. Med.*, 92, 410, 1956.
32. **Moscona, A. A.,** Patterns and mechanisms of tissue reconstruction from dissociated cells, in *Developing Cell Systems and Their Control*, Rudnick, D., Ed., Academic Press, New York, 1960, 45.
33. **Trinkaus, J. P. and Groves, P. W.,** Differentiation in culture of mixed aggregates of dissociated tissue cells, *Proc. Natl. Acad. Sci. U.S.A.*, 41, 787, 1955.
34. **Ansevin, K. D. and Lipps, B. V.,** Histogenesis by cells from embryonic and hatched chicks in giant, plate-like aggregates cultured on a porous matrix, *Biol. Bull. (Woods Hole Mass.)*, 145, 463, 1973.
35. **Okada, T. S.,** Immunohistological studies on the reconstruction of nephric tubules from dissociated cells, *J. Embryol. Exp. Morphol.*, 13, 299, 1965.
36. **Abraham, M.,** Processus de réorganisation dans les agrégats formés par cellules des gonades dissociées d'embryon de poulet, *Arch. Anat. Microsc. Morphol. Exp.*, 49, 333, 1960.
37. **Grund, S. K., Pelliniemi, L. J., Paranko, J., Muller, U., and Lakkala-Paranko, T.,** Reaggregates of cells from rat testis resemble developing gonads, *Differentiation*, 32, 135, 1986.
38. **Zenzes, M. T. and Engel, W.,** The capacity of testicular cells of the postnatal rat to reorganize into histotypic structures, *Differentiation*, 20, 157, 1981.
39. **Gayso, L. R. and Palzi, I.,** Cytotactical phenomena in the organization of in vitro cultured gonads, *Acta Biol. Acad. Sci. Hung.*, 17, 255, 1966.
40. **Zenzes, M. T. and Engel, W.,** The capacity of ovarian cells of the postnatal rat to reorganize into histotypic structures, *Differentiation*, 19, 199, 1981.
41. **Fayet, G., Michel-Bechet, M., and Lissitzky, S.,** Thyrotropin-induced aggregation and reorganization into follicles of isolated porcine-thyroid cells in culture. II. Ultrastructural studies, *Eur. J. Biochem.*, 24, 100, 1971.
42. **Hilfer, S. R.,** Cellular interactions in morphogenesis of the thyroid gland, *Am. Zool.*, 8, 273, 1968.
43. **Hilfer, S. R. and Hilfer, E. K.,** Effects of dissociating agents on the fine structure of embryonic chick thyroid cells, *J. Morphol.*, 119, 217, 1966.
44. **Hilfer, S. R., Iszard, L. B., and Hilfer, E. K.,** Follicle formation in the embryonic chick thyroid. II. Reorganization after dissociation, *Z. Zellforsch. Mikrosk. Anat.*, 92, 256, 1968.
45. **Lissitzky, S., Fayet, G., Giraud, A., Verrier, B., and Torresani, J.,** Thyrotrophin-induced aggregation and reorganization into follicles of isolated porcine-thyroid cell. I. Mechanism of action of thyrotrophin and metabolic properties, *Eur. J. Biochem.*, 24, 88, 1971.
46. **Mallette, J. M. and Anthony, A.,** Growth in culture of trypsin dissociated thyroid cells from adult rats, *Exp. Cell Res.*, 41, 642, 1966.
47. **Mauchamp, J., Margotat, A., Chambard, M., Charrier, B., Remy, L., and Michel-Bechet, M.,** Polarity of three-dimensional structures derived from isolated hog thyroid cells in primary culture, *Cell Tissue Res.*, 204, 417, 1979.

48. **Douglas, W. H. J., Moorman, G. W., and Teel, R. W.,** The formation of histotypic structures from monodisperse fetal rat lung cells cultured on a three-dimensional substrate, *In Vitro*, 12, 373, 1976.
49. **Grover, J. W.,** The relation between the embryogenesis of dissociated chick lung cells and their capacity for reaggregation and histogenesis in vitro, *Exp. Cell Res.*, 24, 171, 1961.
50. **Grover, J. W.,** Reaggregation and organotypic redevelopment of dissociated embryonic chick lung cells in short-term culture, *Natl. Cancer Inst. Monogr.*, 11, 35, 1963.
51. **Mc Atee, J. A. and Douglas, W. H. J.,** In vitro histotypic reaggregation of fetal rat lung cells following cryopreservation and storage in liquid nitrogen, *Birth Defects Orig. Artic. Ser.*, 16(2), 73, 1980.
52. **Tao, T.-W.,** Aggregation of dissociated human embryonic cells: interaction of trophoblast with autologous liver and lung, *Exp. Cell Res.*, 36, 275, 1964.
53. **Alwen, J. and Lawn, A. M.,** The reaggregation of adult rat liver cells maintained in vitro, *Exp. Cell Res.*, 89, 197, 1974.
54. **Ansevin, K. D.,** Aggregative and histoformative performance of adult frog liver cells in vitro, *J. Exp. Zool.*, 155, 371, 1964.
55. **Landry, J., Bernier, D., Ouellet, C., Goyette, R., and Marceau, N.,** Spheroidal aggregate culture of rat liver cells: histotypic reorganization, biomatrix deposition, and maintenance of functional activities, *J. Cell Biol.*, 101, 914, 1985.
56. **Garrod, D. R. and Steinberg, M. S.,** Tissue-specific sorting-out in two dimensions in relation to contact inhibition of cell movement, *Nature*, 244, 568, 1973.
57. **Nicol, A. and Garrod, D. R.,** The sorting out of embryonic cells in monolayer, the differential adhesion hypothesis and the non-specificity of cell adhesion, *J. Cell Sci.*, 38, 249, 1979.
58. **Steinberg, M. S. and Garrod, D. R.,** Observations on the sorting-out of embryonic cells in monolayer culture, *J. Cell Sci.*, 18, 385, 1975.
59. **Cau, P., Michel-Bechet, M., and Fayet, G.,** Morphogenesis of thyroid follicles in vitro, *Adv. Anat. Embryol. Cell Biol.*, 52(2), 5, 1976.
60. **Steinberg, M. S.,** Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells, *J. Exp. Zool.*, 173, 395, 1970.
61. **Seeds, N. W. and Vatter, A. E.,** Synaptogenesis in reaggregating brain cell culture, *Proc. Natl. Acad. Sci. U.S.A.*, 68, 3219, 1971.
62. **Crain, S. M. and Bornstein, M. B.,** Organotypic bioelectric activity in cultured reaggregates of dissociated rodent brain cells, *Science*, 176, 182, 1972.
63. **Morris, J. E. and Moscona, A. A.,** Induction of glutamine synthetase in embryonic retina: its dependence on cell interactions, *Science*, 167, 1736, 1970.
64. **Seeds, N. W.,** Neuronal differentiation in reaggregate cell cultures, *Adv. Cell. Neurobiol.*, 4, 57, 1983.
65. **Armstrong, P. B.,** Modulation of tissue affinities of cardiac myocyte aggregates by mesenchyme, *Dev. Biol.*, 64, 60, 1978.
66. **Bresch, D.,** Recherches préliminaires sur des associations d'organes embryonnaires de poulet en culture *in vitro*, *Bull. Biol.*, 89, 179, 1955.
67. **Holtfreter, J.,** Tissue affinity, a means of embryonic morphogenesis, *Arch. Exp. Zellforsch. Besonders Gewebezuecht.*, 23, 169, 1939. (English translation in *Foundations of Experimental Embryology*, Willier, B. H. and Oppenheimer, J. M., Eds., Prentice-Hall, Englewood Cliffs, N.J., 1964, 186).
68. **Steinberg, M. S.,** Reconstitution of tissues by dissociated cells, *Science*, 141, 401, 1963.
69. **Townes, P. and Holtfreter, J.,** Directed movements and selective adhesion of embryonic amphibian cells, *J. Exp. Zool.*, 128, 53, 1955.
70. **Steinberg, M. S., Armstrong, P. B., and Granger, R. E.,** On the recovery of adhesiveness by trypsin-dissociated cells, *J. Membr. Biol.*, 13, 97, 1973.
71. **Auerbach, R. and Grobstein, C.,** Inductive interaction of embryonic tissues after dissociation and reaggregation, *Exp. Cell Res.*, 15, 384, 1958.
72. **Ikushima, N.,** Development of the organized embryo from the temporarily disaggregated gastrula in amphibia, *Mem. Coll. Sci. Kyoto*, 26, 241, 1959.
73. **Poodry, C. A., Bryant, P. J., and Schneiderman, H. A.,** The mechanism of pattern reconstruction by dissociated imaginal discs of *Drosophila melanogaster*, *Dev. Biol.*, 26, 464, 1971.
74. **Armstrong, P. B.,** A fine structural study of adhesive cell junctions in heterotypic cell aggregates, *J. Cell Biol.*, 47, 197, 1970.
75. **Armstrong, P. B.,** Light and electron microscope studies of cell sorting in combinations of chick embryo neural retina and retinal pigment epithelium, *Wilhelm Roux Arch. Dev. Biol.*, 168, 125, 1971.
76. **Trinkaus, J. P.,** Affinity relationships in heterotypic cell aggregates. La Culture Organotypique, *Colloq. Int. C.N.R.S.*, 101, 209, 1961.
77. **Trinkaus, J. P.,** Behavior of dissociated retinal pigment cells in heterotypic cell aggregates, *Ann. N.Y. Acad. Sci.*, 100, 413, 1963.

78. **Trinkaus, J. P. and Lentz, J. P.,** Direct observation on type-specific segregation in mixed cell aggregates, *Dev. Biol.*, 9, 115, 1964.
79. **Armstrong, P. B. and Armstrong, M. T.,** A role for fibronectin in cell sorting, *J. Cell Sci.*, 69, 179, 1984.
80. **Trinkaus, J. P. and Gross, M. C.,** The use of tritiated thymidine for marking migratory cells, *Exp. Cell Res.*, 24, 52, 1961.
81. **Wiseman, L. L., Steinberg, M. S., and Phillips, H. M.,** Experimental modulation of intercellular cohesiveness: reversal of tissue assembly, *Dev. Biol.*, 28, 498, 1972.
82. **Zwilling, E.,** Survival and non-sorting of nodal cells following dissociation and reaggregation of definitive streak chick embryo, *Dev. Biol.*, 7, 642, 1963.
83. **Burdick, M. L.,** Cell sorting out according to species in aggregates containing mouse and chick embryonic limb mesoblast cells, *J. Exp. Zool.*, 175, 357, 1970.
84. **Burdick, M. L.,** Differences in the morphogenetic properties of mouse and chick embryonic liver cells, *J. Exp. Zool.*, 180, 117, 1972.
85. **Garber, B. B.,** Brain histogenesis in vitro: reconstruction of brain tissue from dissociated cells, *In Vitro*, 8, 167, 1972.
86. **Garber, B. B. and Moscona, A. A.,** Reconstruction of brain tissue from cell suspension. I. Aggregation patterns of cells dissociated from different regions of the developing brain, *Dev. Biol.*, 27, 217, 1972.
87. **Moscona, A.,** Development in vitro of chimeric aggregates of dissociated embryonic chick and mouse cells, *Proc. Natl. Acad. Sci. U.S.A.*, 43, 184, 1957.
88. **Overton, J.,** Formation of junctions and cell sorting in aggregates of chick and mouse cells, *Dev. Biöl.*, 55, 103, 1977.
89. **Abercrombie, M.,** Contact inhibition: the phenomenon and its biological implications, *Natl. Cancer Inst. Monogr.*, 26, 249, 1967.
90. **Armstrong, P. B. and Parenti, D.,** Cell sorting in the presence of cytochalasin B, *J. Cell Biol.*, 55, 542, 1972.
91. **Maslow, D. E. and Mayhew, E.,** Cytochalasin B prevents specific sorting of reaggregating embryonic cells, *Science*, 177, 281, 1972.
92. **Overton, J. and Kapmarski, R.,** Hybrid desmosomes in aggregated chick and mouse cells, *J. Exp. Zool.*, 192, 33, 1975.
93. **Sanger, J. W. and Holtzer, H.,** Cytochalasin B: effects on cell morphology, cell adhesion and muco-polysaccharide synthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 253, 1972.
94. **Steinberg, M. S. and Wiseman, L. L.,** Do morphogenetic tissue rearrangements require active cell movements? The reversible inhibition of cell sorting and tissue spreading by cytochalasin B, *J. Cell Biol.*, 55, 606, 1972.
95. **Overton, J.,** Selective formation of desmosomes in chick cell reaggregates, *Dev. Biol.*, 39, 210, 1974.
96. **Edelstein, B. R.,** Cell specific diffusion model of morphogenesis, *J. Theor. Biol.*, 30, 515, 1971.
97. **Stefanelli, A., Zacchei, A. M., and Ceccherini, V.,** Riconstituzioni retiniche in vitro dopo disgregazione dell'abbozzo oculare di embrione di pollo, *Acta Embryol. Morphol. Exp.*, 4, 47, 1961.
98. **Curtis, A. S. G.,** Timing mechanisms in the specific adhesion of cells, *Exp. Cell Res.*, Suppl. 8, 107, 1961.
99. **Curtis, A. S. G.,** On the occurrence of specific adhesion between cells, *J. Embryol. Exp. Morphol.*, 23, 253, 1970.
100. **Elton, R. A. and Tickle, C. A.,** The analysis of spatial distributions in mixed cell populations: a statistical method for detecting sorting out, *J. Embryol. Exp. Morphol.*, 26, 135, 1971.
101. **Curtis, A. S. G.,** Cell positioning, in *Specificity of Embryological Interactions*, Ser. B, Vol. 4, Garrod, D. R., Ed., Chapman and Hall, London, 1978, 158.
102. **Harris, A. K.,** Is cell sorting caused by differences in the work of intercellular adhesion? A critique of the Steinberg hypothesis, *J. Theor. Biol.*, 61, 267, 1976.
103. **Curtis, A. S. G.,** The behavior of cell populations: model systems, in *The Cell Surface: Its Molecular Role in Morphogenesis*, Logos Press, London, 1967, 222.
104. **Sigot, M.,** *In vitro* reassociation of dissociated cells, in *Organ Culture*, Thomas, J. A., Ed., Academic Press, New York, 1970, 343.
105. **Boyse, E. A.,** Organization of antigenic structures on cell surfaces, in *Biomembranes*, Vol. 2, Manson, L. A., Ed., Plenum Press, New York, 1971, 221.
106. **Moscona, A. A.,** Cell recognition in embryonic morphogenesis and the problem of neuronal specificities, in *Neuronal Recognition*, Barondes, S. H., Ed., Plenum Press, New York, 1976, 205.
107. **Weiss, P.,** The problem of specificity in growth and development, *Yale J. Biol. Med.*, 19, 235, 1947.
108. **Phillips, H. M. and Steinberg, M. S.,** Equilibrium measurements of embryonic chick cell adhesiveness. I. Shape equilibrium in centrifugal fields, *Proc. Natl. Acad. Sci. U.S.A.*, 64, 121, 1969.

109. **Phillips, H. M. and Steinberg, M. S.,** Embryonic tissues as elasticoviscous liquids. I. Rapid and slow shape changes in centrifuged cell aggregates, *J. Cell Sci.*, 30, 1, 1978.
110. **Phillips, H. M., Steinberg, M. S., and Lipton, B. H.,** Embryonic tissues as elasticoviscous liquids. II. Direct evidence for cell slippage in centrifuged aggregates, *Dev. Biol.*, 59, 124, 1977.
111. **Heintzelman, K. F., Phillips, H. M., and Davis, G. S.,** Liquid-tissue behavior and differential cohesiveness during chick limb budding, *J. Embryol. Exp. Morphol.*, 47, 1, 1978.
112. **Phillips, H. M. and Davis, G. S.,** Liquid-tissue mechanics in amphibian grastrulation: germ-layer assembly in *Rana pipiens*, *Am. Zool.*, 18, 81, 1978.
113. **Phillips, H. M.,** Physical analysis of tissue mechanics in amphibian gastrulation, *Am. Zool.*, 24, 657, 1984.
114. **Garrod, D. R.,** Desmosomes, cell adhesion molecules and the adhesive properties of cells in tissues, *J. Cell Sci.*, Suppl. 4, 221, 1986.
115. **Mattey, D. L. and Garrod, D. R.,** Mutual desmosome formation between all binary combinations of human, bovine, canine, avian and amphibian cells: desmosome formation is not tissue or species specific, *J. Cell Sci.*, 75, 377, 1985.
116. **Epstein, M. L. and Gilula, N. B.,** A study of communication specificity between cells in culture, *J. Cell Biol.*, 75, 769, 1977.
117. **Hunter, G. K. and Pitts, J. D.,** Non-selective junctional communication between some different mammalian cell types in primary culture, *J. Cell Sci.*, 49, 163, 1981.
118. **Overton, J.,** Is there an instance of Steinberg's "site frequency model"?, *J. Theor. Biol.*, 65, 787, 1977.
119. **Edelman, G. M.,** Cell adhesion and the molecular processes of morphogenesis, *Annu. Rev. Biochem.*, 54, 135, 1985.
120. **Rutishauser, U., Thiery, J.-P., Brackenbury, R., and Edelman, G. M.,** Adhesion among neural cells of the chick embryo. III. Relationships of the surface molecule CAM to cell adhesion and the development of histotypic patterns, *J. Cell Biol.*, 79, 371, 1978.
121. **Edelman, G. M.,** Cell adhesion molecules, *Science*, 219, 450, 1983.
122. **Edelman, G. M.,** Cell adhesion and morphogenesis: the regulator hypothesis, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 1460, 1984.
123. **Thiery, J.-P., Duband, J.-L., Rutishauser, U., and Edelman, G.,** Cell adhesion molecules in early chicken embryogenesis, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 6737, 1982.
124. **Armstrong, P. B.,** Role of the extracellular matrix in the control of cell motility in a model morphogenetic system, *Prog. Clin. Biol. Res.*, 151, 309, 1984.
125. **Armstrong, P. B.,** Cell recognition, the extracellular matrix and heart morphogenesis, *Exp. Biol. Med.*, 10, 222, 1985.
126. **Armstrong, P. B. and Armstrong, M. T.,** Regulation of tissue patterning in the developing heart by fibronectin, *Prog. Clin. Biol. Res.*, 217B, 177, 1986.
127. **Yamada, K. M., Yamada, S., and Pastan, I.,** The major cell surface glycoprotein of chick embryo fibroblasts is an agglutinin, *Proc. Nat. Acad. Sci. U.S.A.*, 72, 3158, 1975.
128. **Yamada, K. M.,** Cell surface interaction with extracellular materials, *Annu. Rev. Biochem.*, 52, 761, 1983.
129. **Armstrong, P. B. and Niederman, R.,** Reversal of tissue position after cell sorting, *Dev. Biol.*, 28, 518, 1972.
130. **Mc Clay, D. R. and Baker, S. R.,** A kinetic study of embryonic cell adhesion, *Dev. Biol.*, 43, 109, 1975.
131. **Mc Guire, E. J.,** Intercellular adhesive selectivity. II. Properties of embryonic chick liver cell-cell adhesion, *J. Cell Biol.*, 68, 90, 1976.
132. **Mc Guire, E. J. and Burdick, C. L.,** Intercellular adhesive selectivity. I. An improved assay for the measurement of embryonic cell intercellular adhesion (liver and other tissues), *J. Cell Biol.*, 68, 80, 1976.
133. **Roth, S.,** Studies on intercellular adhesive selectivity, *Dev. Biol.*, 18, 602, 1968.
134. **Roth, S., Mc Guire, E. J., and Roseman, S.,** An assay for intercellular adhesive specificity, *J. Cell Biol.*, 51, 525, 1971.
135. **Roth, S. A. and Weston, J. A.,** The measurement of intercellular adhesion, *Proc. Natl. Acad. Sci. U.S.A.*, 58, 974, 1967.
136. **Buultjens, T. E. J. and Edwards, J. G.,** Adhesive selectivity is exhibited *in vitro* by cells from adjacent tissues of the embryonic chick retina, *J. Cell Sci.*, 23, 101, 1977.
137. **Walther, B. T., Öhman, R., and Roseman, S.,** A quantitative assay for intercellular adhesion, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 1569, 1973.
138. **Moyer, W. A. and Steinberg, M. S.,** Do rates of intercellular adhesion measure the cell affinities reflected in cell sorting and tissue-spreading configurations?, *Dev. Biol.*, 52, 246, 1976.
139. **Bernfield, M. R. and Cassiman, J. J.,** Tissue-specific differences and membrane site mobility in intercellular adhesion, in *Extracellular Matrix Influences on Gene Expression*, Slavkin, H. C. and Grenlich, R. C., Eds., Academic Press, New York, 1975, 457.

140. **Cassiman, J. J. and Bernfield, M. R.,** Use of preformed cell aggregates and layers to measure tissue-specific differences in intercellular adhesion, *Dev. Biol.*, 52, 231, 1976.
141. **Cassiman, J. J. and Bernfield, M. R.,** Transformation-induced alterations in adhesion. Binding of preformed cell aggregates to cell layers, *Exp. Cell Res.*, 103, 311, 1976.
142. **Steinberg, M. S.,** The problem of adhesive selectivity in cellular interactions, in *Cellular Membranes in Development,* Locke, M., Ed., Academic Press, New York, 1964, 231.
143. **Glabe, C. G., Grabel, L. B., Vacquier, V. D., and Rosen, S. D.,** Carbohydrate specificity of sea urchin sperm bindin: a cell surface lectin mediating sperm-egg adhesion, *J. Cell Biol.*, 94, 123, 1982.
144. **Lopez, L. C., Bayna, E. M., Litoff, D., Sharper, N. L., Sharper, J. H., and Shur, B. D.,** Receptor function of mouse sperm surface galactosyltransferase during fertilization, *J. Cell Biol.*, 101, 1501, 1985.
145. **Vacquier, V. D. and Moy, G. W.,** Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2456, 1977.
146. **Wassarman, P. M., Florman, H. M., and Greve, J. M.,** Receptor-mediated sperm-egg interactions in mammals, in *Biology of Fertilization,* Vol. 2, Metz, C. B. and Monroy, A., Eds., Academic Press, New York, 1985, 341.
147. **Gallatin, M., St. John, T. P., Siegelman, M., Reichert, R., Butcher, E. C., and Weissman, I. L.,** Lymphocyte homing receptors, *Cell,* 44, 673, 1986.
148. **Siegelman, M., Bond, M. W., Gallatin, W. M., St. John, T., Smith, H. T., Fried, V. A., and Weissman, I. L.,** Cell surface molecule associated with lymphocyte homing is a ubiquitinated branched-chain glycoprotein, *Nature,* 231, 823, 1986.
149. **Stoolman, L. M., Tenforde, T. S., and Rosen, S. D.,** Phosphomannosyl receptors may participate in the adhesive interaction between lymphocytes and high endothelial venules, *J. Cell Biol.*, 99, 1535, 1984.
150. **Yednock, T. A., Stoolman, L. M., and Rosen, S. D.,** Phosphomannosyl-derivatized beads detect a receptor involved in lymphocyte homing, *J. Cell Biol.*, 104, 713, 1987.
151. **Yednock, T. A., Butcher, E. C., Stoolman, L. M., and Rosen, S. D.,** Receptors involved in lymphocyte homing: relationship between a carbohydrate-binding receptor and the MEL-14 antigen, *J. Cell Biol.*, 104, 725, 1987.
152. **Vaysse, J., Gattegno, L., Bladier, D., and Aminoff, D.,** Adhesion and erythrophagocytosis of human senescent erythrocytes by autologous monocytes and their inhibition by B-galactosyl derivatives, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 1339, 1986.
153. **Shur, B. D. and Roth, C.,** Cell surface glycosyltransferrases, *Biochim. Biophys. Acta,* 415, 473, 1975.
154. **Goel, N. S. and Leith, A. G.,** Self-sorting of anisotropic cells, *J. Theor. Biol.*, 28, 469, 1970.
155. **Armstrong, P. B., Roberson, M. M., Nuccitelli, R., and Kline, D.,** Adhesion and polarity of amphibian embryo blastomeres, *Prog. Clin. Biol. Res.*, 85B, 235, 1982.
156. **Sabatini, D. D., Griepp, E. B., Rodriguez-Boulan, E. J., Dolan, W. J., Robbins, E. S., Papadopoulas, S., Ivanov, I. E., and Rindler, M. J.,** Biogenesis of epithelial polarity, in *Modern Cell Biology,* Vol. 2, McIntosh, J. R., Ed., Alan R. Liss, New York, 1985, 419.
157. **Simons, K. and Fuller, S. D.,** Cell surface polarity in epithelia, *Annu. Rev. Cell Biol.*, 1, 243, 1985.
158. **Chen, J., Stuckey, E. C., and Berry, C. L.,** Three-dimensional culture of rat exocrine pancreatic cells using collagen gels, *Br. J. Exp. Pathol.*, 66, 551, 1985.
159. **Herzog, V. and Miller, F.,** Structural functional polarity of inside-out follicles prepared from pig thyroid gland, *Eur. J. Cell Biol.*, 24, 74, 1981.
160. **Nitsch, L., Tacchetti, C., Tramontano, D., and Ambesi-Impiombato, F. S.,** Suspension culture reveals a morphogenetic property of a thyroid epithelial cell line, *Exp. Cell Res.*, 152, 22, 1984.
161. **Wohlwend, A., Montesano, R., Vassalli, J.-D., and Orci, L.,** LLC-PK, cysts: a model for the study of epithelial polarity, *J. Cell. Physiol.*, 125, 533, 1985.
162. **Madri, J. A., Williams, S. K., Wyatt, T., and Mezzio, C.,** Capillary endothelial cell cultures: phenotypic modulation by matrix components, *J. Cell Biol.*, 97, 153, 1983.
163. **Montesano, R., Orci, L., and Vassalli, P.,** In vitro rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices, *J. Cell Biol.*, 97, 1648, 1983.
164. **Folkman, J. and Haudenschild, C.,** Angiogenesis in vitro, *Nature,* 288, 551, 1980.
165. **Feder, J., Marasa, J. C., and Olander, J. V.,** The formation of capillary-like tubes by calf aortic endothelial cells grown in vitro, *J. Cell Physiol.*, 116, 1, 1983.
166. **Chambard, M., Gabrion, J., and Mauchamp, J.,** Influence of collagen gel on the orientation of epithelial cell polarity: follicle formation from isolated thyroid cells and from preformed monolayers, *J. Cell Biol.*, 91, 157, 1981.
167. **Saier, M. H.,** An established kidney epithelial cell line with potential for tissue regeneration, *J. Supramol. Struct.*, Suppl. 5, 301, 1981.
168. **Barriere, H., Chambard, M., Mauchamp, J., and Gabion, J.,** Polarity reversal of inside-out thyroid follicles cultured within collagen gel: an ultrastructural study, *Biol. Cell,* 57, 39, 1986.
169. **Chambard, M., Veirier, B., Gabrion, J., and Mauchamp, J.,** Polarity reversal of inside-out thyroid follicles cultured within collagen gel: reexpression of specific functions, *Biol. Cell,* 51, 315, 1984.

170. **Garbi, C., Nitsch, L., and Wollman, S. H.,** Embedding in a collagen gel stabilizes the polarity of epithelial cells in thyroid follicles in suspension culture, *Exp. Cell Res.*, 151, 458, 1984.
171. **Garbi, C., Tacchetti, C., and Wollman, S. H.,** Change of inverted thyroid follicle into a spheroid after embedding in a collagen gel, *Exp. Cell Res.*, 163, 63, 1986.
172. **Garbi, C. and Wollman, S. H.,** Ultrastructure and some other properties of inverted thyroid follicles in suspension culture, *Exp. Cell. Res.*, 138, 343, 1982.
173. **Garbi, C. and Wollman, S. H.,** Basal lamina formation on thyroid epithelia in separated follicles in suspension culture, *J. Cell Biol.*, 94, 489, 1982.
174. **Davis, G. S.,** Migration-directed liquid properties of embryonic amphibian tissues, *Am. Zool.*, 24, 649, 1984.
175. **Holtfreter, J.,** Properties and functions of the surface coat in amphibian embryos, *J. Exp. Zool.*, 93, 251, 1943.
176. **Holtfreter, J.,** A study of the mechanics of gastrulation. I, *J. Exp. Zool.*, 94, 261, 1943.
177. **Holtfreter, J.,** A study of the mechanics of gastrulation. II, *J. Exp. Zool.*, 95, 171, 1944.
178. **Roberson, M. M., Armstrong, J., and Armstrong, P. B.,** Adhesive and non-adhesive membrane domains of amphibian embryo cells, *J. Cell Sci.*, 44, 19, 1980.
179. **Burnside, B.,** Experimental induction of microfilament formation and contraction, *J. Cell Biol.*, 55, 33a, 1972.
180. **De Ridder, L., Mareel, M., and Vakaet, L.,** Adhesion of malignant and nonmalignant cells to cultured embryonic substrata, *Cancer Res.*, 35, 3164, 1975.
181. **Burgoyne, P. S. and Ducibella, T.,** Changes in the properties of the developing trophoblast as revealed by aggregation studies, *J. Embryol. Exp. Morphol.*, 40, 143, 1977.
182. **Trinkaus, J. P.,** Role of the periblast in Fundulus epiboly, *Ontogenesis*, 2, 401, 1971.
183. **Buck, R. C.,** Walker 256 tumor implantation in normal and injured peritoneum studied by electron microscopy, scanning electron microscopy, and autoradiography, *Cancer Res.*, 33, 3181, 1973.
184. **Di Pasquale, A. and Bell, P. B.,** The upper cell surface: its inability to support active cell movement in culture, *J. Cell Biol.*, 62, 198, 1974.
185. **Elsdale, T. and Bard, J.,** Cellular interactions in morphogenesis of epithelial mesenchymal systems, *J. Cell Biol.*, 63, 343, 1974.
186. **Middleton, C. A.,** The control of epithelial cell locomotion in tissue culture, in *Ciba Foundation Symposium on the Locomotion of Tissue Cells*, Porter, R., Ed., Elsevier, Amsterdam, 1973, 251.
187. **Vasiliev, J. M., Gelfand, I. M., Domnina, L. V., Zacharova, O. S., and Ljubimov, A. V.,** Contact inhibition of phagocytosis in epithelial sheets: alterations of cell surface properties induced by cell-cell contacts, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 719, 1975.
188. **Cowin, P., Mattey, D. L., and Garrod, D. R.,** Distribution of desmosomal components in the tissues of vertebrates, studied by fluorescent antibody staining, *J. Cell Sci.*, 66, 119, 1984.
189. **Ishimaru, Y., Hattori, R., Jinyon, C., and Hayashi, H.,** Cell membrane polarity in rat ascites hepatoma cells. Distribution of a cell surface-associated adhesive factor on the cell surfaces, *Cell Tissue Res.*, 240, 353, 1985.
190. **Roberson, M. M. and Armstrong, P. B.,** Carbohydrate-binding component of amphibian embryo cell surfaces: restriction to surface regions capable of cell adhesion, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 3460, 1980.
191. **Sawyer, P. N. and Srinivasan, S.,** The role of electrochemical surface properties in thrombosis at vascular interfaces: cumulative experience of studies in animals and man, *Bull. N.Y. Acad. Med.*, 48, 235, 1972.
192. **Thubrikar, M., Reich, T., and Cadoff, I.,** Study of surface change of the intima and artificial materials in relation to thrombogenicity, *J. Biomech.*, 13, 663, 1980.
193. **Born, G. V. R. and Palinski, W.,** Unusually high concentrations of sialic acids on the surface of vascular endothelia, *Br. J. Exp. Pathol.*, 66, 543, 1985.
194. **Garrod, D. R.,** Formation of desmosomes in polarized and non-polarized epithelial cells: implications for epithelial morphogenesis, *Biochem. Soc. Trans.*, 14, 172, 1986.
195. **Farquhar, M. G. and Palade, G. E.,** Junctional complexes in various epithelia, *J. Cell Biol.*, 17, 375, 1963.
196. **Dan, K.,** Cyto-embryology of echinoderms and amphibia, *Int. Rev. Cytol.*, 9, 321, 1960.
197. **Wolpert, L. and Gustafson, T.,** Studies on the cellular basis of morphogenesis of the sea urchin embryo — the formation of the blastula, *Exp. Cell Res.*, 25, 374, 1961.
198. **Martz, E., Phillips, H. M., and Steinberg, M. S.,** Contact inhibition of overlapping and differential cell adhesion: a sufficient model for the control of certain cell culture morphologies, *J. Cell Sci.*, 16, 401, 1974.
199. **Ingber, D. E., Madri, J. A., and Jamieson, J. D.,** Basement membrane as a spatial organizer of polarized epithelia. Exogenous basement membrane reorients pancreatic epithelial tumor cells *in vitro*, *Am. J. Pathol.*, 122, 129, 1986.

200. **Vracko, R.,** Basal lamina scaffold — anatomy and significance for maintenance of orderly tissue structure, *Am. J. Pathol.*, 77, 314, 1974.
201. **Nitsch, L. and Wollman, S. H.,** Suspension culture of separated follicles consisting of differentiated thyroid epithelial cells, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 472, 1980.
202. **Nitsch, L. and Wollman, S. H.,** Ultrastructure of intermediate stages in polarity reversal of thyroid epithelium in follicles in suspension culture, *J. Cell Biol.*, 86, 875, 1980.
203. **Valentich, J. D., Tchao, R., and Leighton, J.,** Hemicyst formation stimulated by cyclic AMP in dog kidney cell line MDCK, *J. Cell. Physiol.*, 100, 291, 1979.
204. **Slack, C. and Warner, A. E.,** Intracellular and intercellular potentials in the early amphibian embryo, *J. Physiol. (London)*, 232, 313, 1973.
205. **Kimmel, C. B. and Warga, R. M.,** Tissue-specific cell lineages originate in the gastrula of the zebrafish, *Science*, 231, 365, 1986.
206. **Lesseps, R. J., Geurts Van Kessel, A. H. M., and Denuce, J. M.,** Cell patterns and cell movements during early development of an annual fish, *Nothobranchius neumanni*, *J. Exp. Zool.*, 193, 137, 1975.
207. **Trinkaus, J. P.,** Surface activity and locomotion of *Fundulus* deep cells during blastula and gastrula stages, *Dev. Biol.*, 30, 68, 1973.
208. **Wourms, J. P.,** The developmental biology of annual fishes. II. Naturally occurring dispersion and reaggregation of blastomeres during the development of annual fish eggs, *J. Exp. Zool.*, 182, 169, 1972.
209. **Le Douarin, N.,** Cell line segregation during peripheral nervous system ontogeny, *Science*, 231, 1515, 1986.
210. **England, M. A. and Wakely, J.,** Scanning electron microscopy of the development of the mesoderm layer in chick embryos, *Anat. Embryol.*, 150, 291, 1977.
211. **Mayer, B. W. and Packard, D. S.,** A study of the expansion of the chick area vasculosa, *Dev. Biol.*, 63, 335, 1978.
212. **Vogt, W.,** Gestaltungsanalyse am Amphibienkeim mit ortlicher Vitalfärbong. II. Teil. Gastrulation und Mesodermbildong bei Urodelen und Anvren, *Wilhelm Roux Arch. Entwicklungsmech. Org.*, 120, 384, 1929.
213. **Bellairs, D.,** Differentiation of the yolk sac of the chick studied by electron microscopy, *J. Embryol. Exp. Morphol.*, 11, 201, 1963.
214. **Chernoff, E. A. G. and Overton, J.,** Scanning electron microscopy of chick epiblast expansion on the vitelline membrane. Cell-substrate interactions, *Dev. Biol.*, 57, 33, 1977.
215. **Downie, J. R.,** The mechanism of chick blastoderm expansion, *J. Embryol. Exp. Morphol.*, 35, 559, 1976.
216. **New, D. A. T.,** The adhesive properties and expansion of the chick blastoderm, *J. Embryol. Exp. Morphol.*, 7, 146, 1959.
217. **Betchaku, T. and Trinkaus, J. P.,** Programmed endocytosis during epiboly of *Fundulus heteroclitus*, *Am. Zool.*, 26, 193, 1986.
218. **Trinkaus, J. P.,** A study of the mechanism of epiboly in the egg of *Fundulus heteroclitus*, *J. Exp. Zool.*, 118, 269, 1951.
219. **Trinkaus, J. P.,** Mechanism of *Fundulus* epiboly — a current view, *Am. Zool.*, 24, 673, 1984.
220. **Ho, E. and Shimada, Y.,** Formation of the epicardium studied with the scanning electron-microscope, *Dev. Biol.*, 66, 579, 1978.
221. **Manasek, F. J.,** Embryonic development of the heart. II. Formation of the epicardium, *J. Embryol. Exp. Morphol.*, 22, 333, 1969.
222. **Lash, J.,** Studies on wound closure in urodeles, *J. Exp. Zool.*, 128, 13, 1955.
223. **Radice, G. P.,** The spreading of epithelial cells during wound closure in *Xenopus* larvae, *Dev. Biol.*, 76, 26, 1980.
224. **Izzard, C. S. and Lochner, L. R.,** Cell-to-substrate contacts in living fibroblasts: an interference reflexion study with an evaluation of the technique, *J. Cell Sci.*, 21, 129, 1976.
225. **Rees, D. A., Lloyd, C. W., and Thom, D.,** Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins, *Nature*, 267, 124, 1977.
226. **Letourneau, P. C.,** Possible role for cell-to-substratum adhesion in neuronal morphogenesis, *Dev. Biol.*, 44, 77, 1975.
227. **Letourneau, P. C.,** Cell-to-substratum adhesion and guidance of axonal elongation, *Dev. Biol.*, 44, 92, 1975.
228. **Carter, S. B.,** Haptotaxis and the mechanism of cell motility, *Nature*, 213, 256, 1967.
229. **Harris, A.,** Behavior of cultured cells on substrata of variable adhesiveness, *Exp. Cell Res.*, 77, 285, 1973.
230. **Dierich, M. P., Wilhelmi, D., and Till, G.,** Essential role of surface-bound chemoattractant in leukocyte migration, *Nature*, 270, 351, 1977.
231. **Lacovara, J., Cramer, E. B., and Quigley, J. P.,** Fibronectin enhancement of directed migration of B16 melanoma cells, *Cancer Res.*, 44, 1657, 1984.
232. **Mc Carthy, J. B., Palm, S. L., and Furcht, L. T.,** Migration by haptotaxis of a Schwann cell tumor line to the basement membrane glycoprotein laminin, *J. Cell Biol.*, 97, 772, 1983.

233. **Gustafson, T. and Wolpert, L.,** Studies on the cellular basis of morphogenesis in the sea urchin embryo. Directed movements of primary mesenchyme cells in normal and vegetalized larvae, *Exp. Cell Res.*, 24, 64, 1961.
234. **Poole, T. J. and Steinberg, M. S.,** Evidence for the guidance of pronephric duct migration by a craniocaudally traveling adhesion gradient, *Dev. Biol.*, 92, 144, 1982.
235. **Sidman, R. L. and Rakic, P.,** Neuronal migration, with special reference to developing human brain: a review, *Brain Res.*, 62, 1, 1973.
236. **Hoffman, S., Friedlander, D. R., Chuong, C.-M., Grumet, M., and Edelman, G. R.,** Differential contributions of Ng-CAM and N-CAM to cell adhesion in different neural regions, *J. Cell Biol.*, 103, 145, 1986.
237. **Lindner, J., Rathjen, F. G., and Schachner, M.,** L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum, *Nature*, 305, 427, 1983.
238. **Carlson, B. M.,** *Patten's Foundations of Embryology*, 4th ed., McGraw-Hill, New York, 1981.
239. **Hopper, A. F. and Hart, N. H.,** *Foundations of Animal Development*, 2nd ed., Oxford University Press, Oxford, 1985.
240. **Spemann, H.,** *Embryonic Development and Induction*, Yale University Press, New Haven, CT, 1938.
241. **Eyal-Giladi, H., Kochav, S., and Yerushalmi, S.,** The sorting out of thymidine-labelled chick hypoblast cells in mixed epiblast-hypoblast aggregates, *Differentiation*, 4, 57, 1975.
242. **Steinberg, M. S. and Poole, T. J.,** Strategies for specifying form and pattern: adhesion-guided multicellular assembly, *Philos. Trans. R. Soc. London Ser. B*, 295, 451, 1981.
243. **Steinberg, M. S. and Poole, T. J.,** Liquid behavior of embryonic tissues, in *Cell Behaviour*, Bellairs, R., Curtis, A. S. G., and Dunn, G., Eds., Cambridge University Press, Cambridge, 1982, 583.
244. **Steinberg, M. S. and Poole, T. J.,** Cellular adhesive differentials as determinants of morphogenetic movements and organ segregation, in *Developmental Order: Its Origin and Regulation*, Subtelny, S. and Green, P. B., Eds., Alan R. Liss, New York, 1982, 351.
245. **Searls, R. L.,** Segregation of cells that differentiated without cell movement from a single precursor population, *Exp. Cell Res.*, 64, 163, 1971.
246. **Searls, R. L.,** Cellular segregation: a "late" differentiative characteristic of chick limb bud cartilage cells, *Exp. Cell Res.*, 73, 57, 1972.
247. **Abercrombie, M.,** Contact inhibition in tissue culture, *In Vitro*, 6, 128, 1970.
248. **Allison, F., Smith, M. R., and Wood, W. B.,** Studies on the pathogenesis of acute inflammation. I. The inflammatory reaction to thermal injury as observed in the rabbit ear chamber, *J. Exp. Med.*, 102, 655, 1955.
249. **Clark, E. R. and Clark, E. L.,** Microscopic observations on the growth of blood capillaries in the living mammal, *Am. J. Anat.*, 64, 251, 1939.
250. **Clark, E. R., Clark, E. L., and Rex, R. O.,** Observation on polymorphonuclear leukocytes in the living animal, *Am. J. Anat.*, 59, 123, 1936.
251. **Gabbiani, G.,** The role of contractile proteins in wound healing and fibrocontractive diseases, *Methods Achiev. Exp. Pathol.*, 9, 187, 1979.
252. **Jennings, M. A. and Florey, H. W.,** Healing, in *General Pathology*, 4th ed., Florey, H. W., Ed., W. B. Saunders, Philadelphia, 1970, 480.
253. **Armstrong, P. B.,** Cellular positional stability and intercellular invasion, *BioScience*, 27, 803, 1977.
254. **Armstrong, P. B.,** Invasiveness of non-malignant cells, in *Invasion: Experimental and Clinical Implications*, Mareel, M. M. and Calman, K. C., Eds., Oxford University Press, Oxford, 1984, 120.
255. **Armstrong, P. B.,** Stabilization of tissue architecture: involvement of the extracellular matrix, *Prog. Clin. Biol. Res.*, 171, 87, 1985.
256. **Chen, L. B., Gallimore, P. H., and Mcdougall, J. K.,** Correlation between tumor induction and the large external transformation sensitive protein on the cell surface, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 3570, 1976.
257. **Ruoslahti, E.,** Fibronectin in cell adhesion and invasion, *Cancer Metast. Rev.*, 3, 43, 1984.
258. **Sperry, R.,** Chemoaffinity in the orderly growth of nerve fiber patterns and connections, *Proc. Natl. Acad. Sci. U.S.A.*, 50, 703, 1963.
259. **Fraser, S. E.,** Cell interactions involved in neuronal patterning: an experimental and theoretical approach, in *Molecular Basis of Neural Development*, Edelman, G. M., Gall, W. E., and Cowan, W. M., Eds., Wiley, New York, 1985, 481.
260. **Takeichi, M.,** Functional correlation between cell adhesive properties and some cell surface proteins, *J. Cell Biol.*, 75, 464, 1977.
261. **Thomas, W. A.,** Dual adhesive recognition systems in chick embryonic cells, in *Developmental Biology: A Comprehensive Synthesis*, Vol. 3, Steinberg, M. S., Ed., Plenum Press, New York, 1986, 157.
262. **Takeichi, M., Atsumi, T., Yoshida, C., Uno, K., and Okada, T. S.,** Selective adhesion of embryonal carcinoma cells and differentiated cells by Ca^{2+}-dependent sites, *Dev. Biol.*, 87, 340, 1981.

263. **Takeichi, M., Wzaki, H. S., Tokunaga, K., and Okada, T. S.,** Experimental manipulation of cell surface to affect cellular recognition mechanisms, *Dev. Biol.*, 70, 195, 1979.
264. **Takeichi, M., Shirayoshi, Y., Hatta, K., and Nose, A.,** Cadherins: their morphogenetic role in animal development, *Prog. Clin. Biol. Res.*, 217B, 17, 1986.
265. **Thiery, J. P., Delouvee, A., Gallin, W. J., Cunningham, B. A., and Edelman, G. M.,** Ontogenetic expression of cell adhesion molecules: L-CAM is found in epithelia derived from the three primary germ layers, *Dev. Biol.*, 102, 61, 1984.
266. **Nardi, J. B. and Kafatos, F.,** Polarity and gradients in lepidopteran wing epidermis. II. The differential adhesiveness model: gradient of a non-diffusible cell surface parameter, *J. Embryol. Exp. Morphol.*, 36, 489, 1976.
267. **Nubler-Jung, K.,** Pattern stability in the insect segment. I. Pattern reconstitution by intercalary regeneration and cell sorting in *Dysdercus intermediumn* Dist, *Wilhelm Roux' Arch. Dev. Biol.*, 183, 17, 1977.
268. **Nubler-Jung, K.,** Pattern stability in the insect segment. II The intersegmental region, *Wilhelm Roux' Arch. Dev. Biol.*, 186, 211, 1979.
269. **Mittenthal, J. E. and Mayo, R. M.,** A model for shape generation by strain and cell-cell adhesion in the epithium of an arthropod leg segment, *J. Theor. Biol.*, 100, 443, 1983.
270. **Nardi, J. B. and Stocum, D. L.,** Surface properties of regenerating limb cells: evidence for gradation along the proximodistal axis, *Differentiation*, 25, 27, 1983.
271. **Stocum, D. L.,** The urodele limb regeneration blastema. Determination and organization of the morphogenetic field, *Differentiation*, 27, 13, 1984.
272. **Ruoslahti, E.,** Fibronectin and its receptors, *Annu. Rev. Biochem.*, 57, 375, 1988.
273. **Ruoslahti, E. and Pierschbacher, M. D.,** New perspectives in cell adhesion: RGD and integrins, *Science*, 238, 491, 1987.