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Reconstruction of Tissues by Dissociated Cells

Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation.

Malcolm S. Steinberg

How is the structure of a multicellular animal generated? In the broadest terms, we can distinguish three kinds of developmental processes: growth, differentiation, and morphogenesis. The developing organism multiplies its cells and increases its mass. The emergent parts become different—different from what they were before and different from one another. And the differentiating parts bend inward or outward, expand, contract, disperse, condense, fuse, separate, elongate, even perish, and otherwise rearrange themselves in the process of constructing the animal. But what are the mechanisms which elicit and orient these tissue movements of morphogenesis?

Background of the Problem

Early workers envisioned the tissue movements as resulting from pressures or other inhomogeneities in the immediate environment, but a considerable body of evidence has meanwhile been accumulated to show that the movements are due to intrinsic properties of the individual tissues themselves. Beyond this statement, however, we find

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ourselves in an area of uncertainty, for the character of these intrinsic properties has not been securely and rigorously established. A crack in the shell surrounding this problem appeared very early. H. V. Wilson discovered, in 1907, that the cells and cell clusters obtained by squeezing a sponge through the meshes of fine, silk, bolting cloth could reunite, and that aggregates obtained in this way could reconstitute themselves into functional sponges (1). The manner in which this reconstitution was effected remained problematical. Wilson continued to maintain (2) that a considerable amount of dedifferentiation and redifferentiation occurred, and that cells altered their cytological characteristics to conform with their newly established environments, while other workers (3) believed they had demonstrated the reconstitution to consist, in large measure, of a sorting out of the various types of cells, each coming again to occupy its accustomed haunts in the body of the sponge. The difficulty lay in the absence of permanent and recognizable characteristics by which one could accurately distinguish and follow the various types of cells during the process of reorganization.

In the meantime, Harrison (4) had laid the foundation for modern neuro-embryology, a foundation which in-

cluded the concepts of the selection of paths by outgrowing nerves and of the specificity of nerve-end organ connection, and which was ably extended and built upon by the researches of P. Weiss, Hamburger, and others (see 5).

A second discovery of major importance appeared against this background in 1939. Holtfreter, working with carefully defined tissue fragments from young amphibian embryos, found that these fragments showed marked preferences in their adhesive properties. These preferences were correlated with their normal morphogenetic functions. For example, ectoderm and endoderm, isolated from a gastrula, would adhere to each other much as they do at the same stage in vivo. In time, however, these two tissues would separate from one another, an event which occurs in the embryo as well. This separation is accomplished, in normal development, by the penetration of the mesoderm between the ectoderm and endoderm. Mesoderm incorporated along with the isolated ectoderm and endoderm was indeed found to bind the latter two tissues together in a permanent union in vitro as it does in vivo. Furthermore, when the tissues were present in the right proportions, the ectoderm would take up an external position and the endoderm an internal position, with the mesoderm spread out in between, duplicating in the culture vessel not only the associations but also the anatomical relations which exist in the embryo. An impressive array of similar results with these and other tissues (6) led Holtfreter to frame the concept of "tissue affinities" to describe these associative preferences, which he had shown to be so closely related to normal morphogenetic events.

A third advance was made by Holtfreter in 1944. He found that by subjecting a fragment of an amphibian gastrula to an environmental pH of about 10, he could cause the individual cells to separate and fall away from one another, much as Herbst had earlier been able to cause the separation of sea

401

urchin blastomeres in calcium-free sea water (7). Upon return to a more neutral pH, the amphibian cells would re-establish mutual adhesions, attaching themselves to any neighbors with which they came into contact, and building, in this manner, masses of tissue into which cells of the various germ layers were incorporated at random. The situation resembled that in the sponges, but with one important distinction. Differences in the degree of pigmentation of the amphibian cells, together with their extraordinarily large size, allowed the investigator to follow the movements at least of the surface cells. Before his eyes the lightly pigmented mesoderm cells vanished into the depths of the tissue mass, while darkly pigmented ectoderm cells and the almost pigment-free endoderm cells emerged to replace them at the periphery (8). Sorting out was a reality. And the tissue affinities which Holtfreter had earlier described could with justice be renamed *cell* affinities, for it was now clear that they were inherent in the individual cells.

Other workers made significant contributions. Principal among these was Moscona, who opened the way for investigations with the cells of older avian embryos through his discovery that trypsin was effective in dissociating their tissues (9). Through the use of this technique, it was shown by Moscona (10) and by Trinkaus and Groves (11) that the reconstitution of body parts by aggregates of intermixed cells occurred even though the constituent cells were, in all likelihood, "determined" with respect to their fates, and even though they had already reached their appropriate positions within the embryo. The same fact was established for older amphibian embryos as well, by Townes and Holtfreter (12). The remarkable degree to which normal structure could be approximated by a "self-organizing" cell mixture was demonstrated by Weiss and Taylor (13), who, by culturing aggregates derived from highly differentiated organs in a site which provided vascularization, obtained organogenesis which strikingly approached the complexity of normal organization.

The foregoing historical account is only a sketch, which makes no pretense of complete coverage. It serves, however, to document the fact that formerly elusive problems concerning the mechanisms of morphogenetic movement have been brought more closely within the experimenter's grasp.

Sorting Out, Adhesion, and Motility

The most fundamental facts concerning tissue reconstruction are perhaps the following. (i) When the cells of different vertebrate embryonic tissues are dissociated and mixed, they are capable of establishing adhesions with one another and constructing common aggregates. (ii) Within such "mixed" aggregates, containing cells from different tissues, the differing kinds of cells regroup, each with the others allied to it, to reconstruct the various tissues of origin. (iii) These tissues are reconstructed in definite positions (see also 11); for example, muscle is always built external to cartilage, never the other way around. (iv) When the tissues employed are parts of a complex within the embryo, the geometry of the entire normal complex is reflected in the re-established structures.

In normal development, tissue X may spread from some previous position to cover the surface of tissue Y. In a mixed aggregate in vitro, the same ultimate geometry would be achieved through the sorting out of the jumbled X and Y cells. The fact that the specific anatomical structure is established by pathways which differ so greatly in the two cases is to be regarded less as a curiosity than as a stroke of great fortune for the student of morphogenesis. It indicates that the features responsible for the ultimate anatomical organization are common to these two disparate systems. In the case at hand, two common features at once come to mind. They are the basic cellular properties of mutual adhesiveness and motility. It is not my purpose here to cover the extensive literature concerning cellular adhesion and cell movement, much of which is discussed in recent publications (14, 15). I wish rather to examine two particular assumptions which, either singly or in combination, are widely held to be necessary in order to account for sorting out and for tissue reconstruction. These assumptions are (i) that the segregating cells exhibit actively directed movements, and (ii) that they display qualitatively selective mutual adhesion.

The segregation of cell species which takes place within mixed aggregates could, in principle, be brought about in either of two ways. Either the differing cells might seek out, by active and directed migration, different parts of the aggregate (or even one another), or they might possess type-specific differences in adhesiveness by virtue of

which the old cellular alliances are again progressively built up through the agency of random collisions. Both possibilities, as well as a combination of the two, have been suggested by various authors. In view of the early experimental documentation by Holtfreter (6, 16) of differences in adhesiveness among such cells, and because of the apparent cellular selectivity involved in wound healing and in neurogenesis, most of the speculation has centered around possible mechanisms by which adhesion might be rendered selective. There is no body of evidence for mutual attraction (or repulsion) by embryonic cells.

It has been variously proposed that embryonic cells selectively adhere to one another by means of binding sites which possess singularities of conformation (5, 17, 18), of chemical composition (19), or of geometric arrangement (5, 14, 17, 20); that adhesion among differing cells is nonselective in character but varies in its intensity as a function of cell type and time (21) or as a result of selective influences which favor disjunction (22); and that in addition to (12), or possibly in lieu of (23), showing selectivity in their adhesion to one another, cells may migrate in a directed fashion either inward or outward within multicellular masses, the migration ultimately bringing about their mutual segregation.

In virtually all the hypotheses which have been advanced, attention has been focused upon the adhesive and motor properties of the segregating cells; for without adhesion there can be no coherent multicellular aggregate, and without motility on the part of the component cells there can be no sorting out. Almost all authors who have dealt with this problem [with the exception of Stefanelli et al. (23)] have in addition assumed that the differences in adhesiveness between the different types of cells are type specific, at least throughout the period during which sorting out occurs. Ample justification for this assumption is to be found in the experimental literature, as I have pointed out. Beyond this point, each additional assumption increases the risk of error.

I wish now to develop the thesis that the behavior that is characteristic of cells in the process of sorting out and of tissue reconstruction follows directly from their possession of motility and of quantitative differentials in adhesiveness, unrestricted by any requirement for qualitative specificity. It will be

helpful in this analysis to review first the behavior of inanimate physical systems which share with living cells precisely these attributes, and to examine the way in which this behavior is influenced by the particular quantitative adhesive relationships which apply. In this way we may see the consequences of the presence of these motor and adhesive properties, unobscured by any of the complex and often seemingly goal-directed activities of which cells are capable.

In the physical world, we recognize that the units which comprise a gas are mobile but not coherent—they fly apart to fill as much space as is provided. When the energy which drives them apart is sufficiently reduced, attractive forces begin to dominate and the units form a different type of system—a liquid, in which they retain mobility but gain coherence. Reduction of the thermal energy to a still lower point results in the domination of attractive forces to such an extent that mobility of the units is effectively inhibited, and we have a system in which coherence is retained but mobility is severely restricted—a solid. Thus, in the world of molecules, a liquid system is one which is composed of a population of coherent, mobile units.

Many of the properties of liquid systems depend exclusively upon this fact. It is of no substantive consequence that the units happen to be molecules and that their motility happens to be passive rather than active in nature. These properties are independent of the composition of the units, independent of the causes of their motility, and independent of the nature of the adhesive forces. For example, a liquid drop assumes a spherical shape when subjected to uniform external conditions, because the mobile units of which it is composed attract or adhere to one another until the greatest possible number have the maximum possible contact. Adhesion being nothing more than close-range attraction, the same holds true for a population of actively motile, uniformly adhesive cells (Fig. 1).

The same principle can be expressed by saying that the *free energy* of the drop reduces to a minimum. Included in this quantity is the *surface free energy*, which provided the impetus in the simple illustration given. The surface free energy is merely the energy available for adhesion but "left over" in the surface, where adhesions could be formed but have not been. It is readily seen to be directly proportional
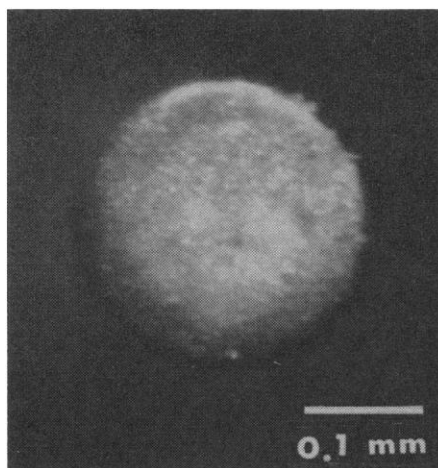
Fig. 1. An initially jagged fragment of liver that has assumed a spherical shape. Isolated from a 5-day chick embryo, it has been maintained in liquid medium at 37°C for 2 days under constant gyration. The same result is obtainable in a stationary culture.

to both (i) the area of exposed surface and (ii) the free energy per unit of surface area, the latter quantity being a direct reflection of the adhesiveness of the units which comprise the surface. The free energy is a potential energy and will tend spontaneously to decrease toward a minimum in *any* population of mobile, coherent units. At this minimum the system is in thermodynamic equilibrium.

Let us now consider the manner in which thermodynamic equilibrium is achieved in a coherent population consisting of two different kinds of mobile units which adhere to one another with different "strengths." The standard measure of the "strength" of such adhesions is called the work of adhesion. This is a measure of the work done by the system in the formation of an adhesion over a unit of area. [In common usage, the term *work of adhesion* refers to adhesion between two different phases while the equivalent term *work of cohesion* refers to adhesion among the units of a single phase (24).] The units adhere to one another, rearranging themselves, as in our first example, until the free energy of the system is reduced to a minimum. This minimum is achieved when the total work done through adhesion in the system is raised to a maximum—in other words, when all of the individual units are mutually oriented in such a manner that they adhere to one another with the greatest average tenacity. At this point of thermodynamic equilibrium, the distribution of the two different types of units (phases) within the system is a function

of the work of cohesion of each of the two phases and of the work of adhesion between them. There are three types of distribution, each corresponding with one of the following three sets of adhesive relationships, in which the two kinds of units are denoted, respectively, as *a* and *b*: (i) *a-b* adhesions equaling or exceeding in strength the average of *a-a* adhesions plus *b-b* adhesions; (ii) *a-b* adhesions weaker than this average but equaling or exceeding in strength the weaker of the other two kinds of adhesions; and (iii) *a-b* adhesions weaker than either the *a-a* or the *b-b* adhesions. Let us now explore the three situations with which these relationships correspond.

We will designate the work of cohesion among the units of type *a* as W_a, the work of cohesion among the units of type *b* as W_b, and the work of adhesion between *a* and *b* units a W_{ab}. If it happens that *a-b* adhesions equal or exceed in strength a value obtained by averaging the strengths of *a-a* adhesions and *b-b* adhesions, we can describe the situation by the relation

$$W_{ab} \geqq \frac{W_a + W_b}{2} \qquad (1)$$

In such a case the greatest average tenacity of adhesion is achieved when *a* and *b* units are alternately arranged in the coherent population, so as to have the maximum possible interconnection. Therefore, at thermodynamic equilibrium, the two populations are intermixed. This is our case 1 (see Fig. 2). If, on the other hand, the strength of the *a-b* adhesions falls below this average value, the situation is described by the opposite relation,

$$W_{ab} < \frac{W_a + W_b}{2} \qquad (2)$$

In this case the greatest average tenacity of adhesion is achieved when *a* and *b* units are totally segregated in the population. However, even the mutual disposition of the segregating *a* and *b* phases is thermodynamically controlled, a fact which is shown as follows.

To begin with, let us establish the convention that when the cohesiveness of the units comprising the two phases differs, the more cohesive units will be designated *a* and the less cohesive units, *b*. Now, if *b* units adhere to *a* units with a tenacity that is equal to or greater than the tenacity with which they adhere to one another, we can express this by the relation

$$W_{ab} \geqq W_b \qquad (3)$$

403

Relations 2 and 3, taken together, determine the complete set of conditions

$$\frac{W_a + W_b}{2} > W_{ab} \geqq W_b \qquad (4)$$

These conditions can only be met when $W_a > W_{ab}$. What are the consequences for the mutual disposition of the segregating phases? Since a-b adhesions are intermediate in strength between a-a and b-b adhesions, the two kinds of units adhere relatively strongly, the whole coherent population tending to assume a spherical form, in which the exposed surface area is minimal. However the surface free energy of the system is the product of the exposed surface area and the free energy per unit of such area, and the free energy per unit area, as we saw earlier, is a measure of the cohesiveness of the units in the surface. Consequently, minimization of the surface free energy is achieved only when the surface is of minimal area and contains exclusively the less cohesive of the two kinds of units which comprise the population. Therefore, in the segregation of the a and b phases, phase b will come to occupy completely the surface of the system, which will, as a whole, tend to assume spherical form. Furthermore, the greatest possible segregation of the two phases will occur, a condition which requires that the interfacial area between the two be minimized. Phase a being totally subsurface, this condition is met when phase a itself assumes the form of a sphere totally enclosed by phase b. Thermodynamic equilibrium is thus established when the less cohesive units are arranged as a coherent sphere totally enclosing a second sphere composed of all of the more cohesive units. This is our case 2 (see Fig. 2).

Only one other set of possible adhesive relationships remains, at this point, to be explored. What will be the disposition of the phases at thermodynamic equilibrium if a-b adhesions, instead of being as strong as, or stronger than, the average of a-a and b-b adhesions (as in our case 1), or weaker than this average but yet as strong as, or stronger than, b-b adhesions (as in our case 2), are the weakest adhesions of all? This circumstance is described by the relationship

$$W_a \geqq W_b > W_{ab} \qquad (5)$$

Let us begin by considering the most extreme possible examples. At the one extreme, a and b units do not adhere to one another at all. Clearly, two separate, isolated populations will form. Each will consist at equilibrium of a sphere containing one of the two types of units. At the other extreme, a and b units adhere to one another with a strength ever so slightly less than that achieved by the adhesion of a pair of b units to one another. Were the a-b adhesions any stronger, they would be equal in strength to the b-b adhesions and we would have at equilibrium the limiting example of case 2: a sphere within a sphere. Instead, the distribution of the phases at equilibrium is
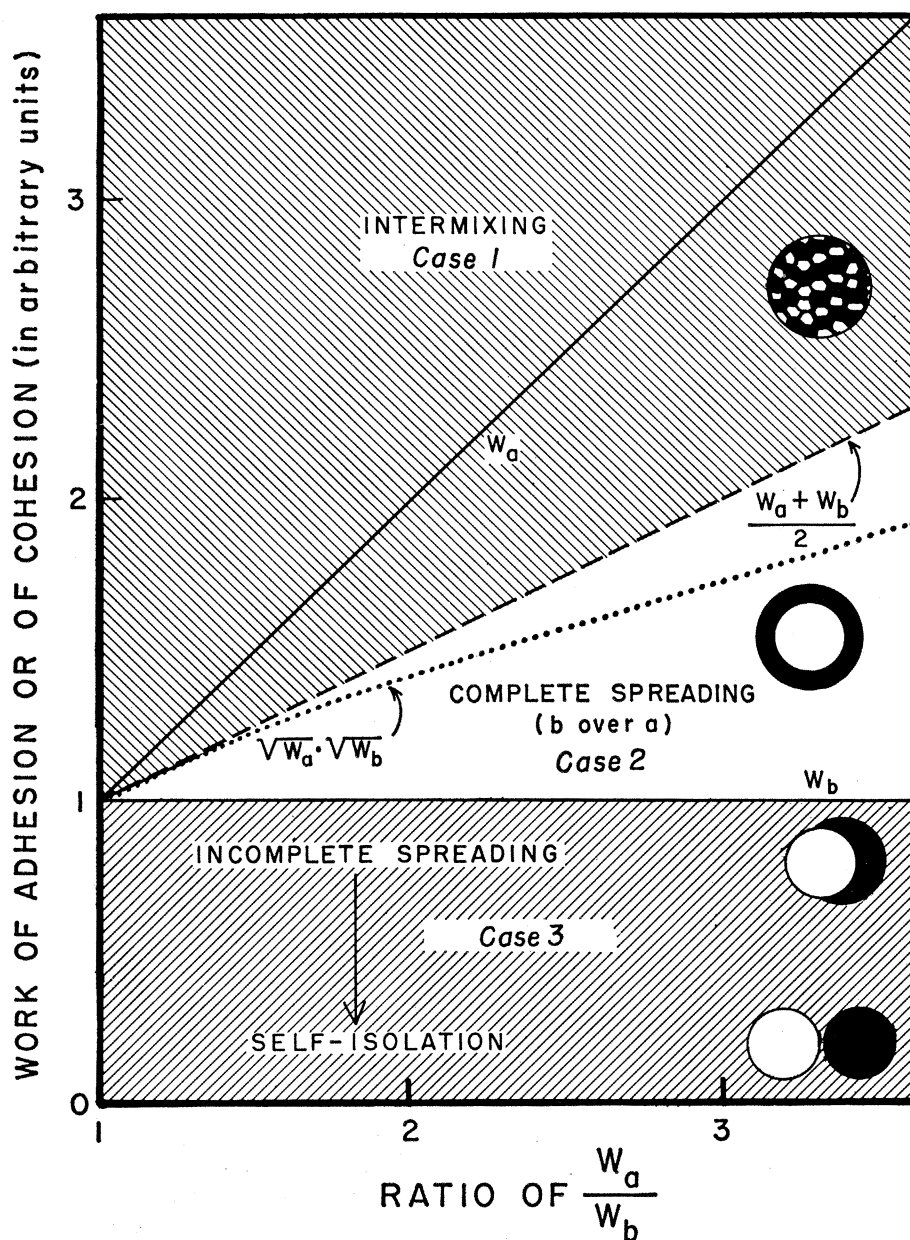

Fig. 2. Types of phase distribution, at equilibrium, in coherent populations consisting of mobile units of two kinds. The work of cohesion of the weakly cohesive b units, arbitrarily assigned a value of 1, is given by the line W_b. The work of cohesion of the more strongly cohesive a units is denoted by W_a. The diagram is used as follows. For any set of adhesive relationships, that vertical line is drawn which passes through the calculated value of W_a/W_b as read on the abscissa. The work of adhesion of a units to b units (W_{ab}), as read on the ordinate, is then entered upon this line. The background shading at this point indicates the distribution of the a and b phases for this system at thermodynamic equilibrium. *Example*: If $W_a = 3$, with W_b defined as 1, then $W_{ab} = 2.1$ would yield intermixing; $W_{ab} = 1.5$ would yield complete coverage of a by b; and $W_{ab} = 0.5$ would yield incomplete coverage of a by b (see *24*). The intersection of the vertical line with the dotted line $(W_a)^{1/2} (W_b)^{1/2}$ marks the value of W_{ab} which would be generated in the model system devoid of adhesive specificity, as described in the text. [Modified from Steinberg (*30*)]

shifted slightly from this configuration in the direction toward isolation of the phases: phase *b* recedes at one spot, exposing a minute area of phase *a* at the periphery (24). The lower the strength of the *a-b* adhesions, the greater the recession, at equilibrium, of the margins of phase *b* around the spherical perimeter of phase *a*. This is our case 3 (see Fig. 2). This type of circumstance is classically described by the relationship known as "Young's equation" (25). Expressed in terms of work of adhesion, this equation becomes

$$2 \frac{W_{ab}}{W_b} = 1 + \cos \theta \qquad (6)$$

where θ represents the internal angle of contact, at equilibrium, of the margin of phase *b* with the surface of phase *a* (see 24, however). It has recently been proved (26) that Young's equation is a "direct consequence of: (1) the existence of interfacial free energies and (2) the total free energy of a system at equilibrium being a minimum." The various possible relationships among W_a, W_b, and W_{ab}, and the topographic relationships which they engender, are shown diagrammatically in Fig. 2. They may be derived from equations presented in most standard texts on surface chemistry (see, for example, 25).

Phase Redistribution in Cohering Populations of Embryonic Cells

The regroupings discussed in the foregoing section are those which tend spontaneously to occur, for thermodynamic reasons, within a population of mobile, mutually adhesive units of two kinds, when the latter are brought into contact. Vertebrate embryonic cells of different kinds are both mobile and mutually adhesive, and they tend, when mixed, to regroup themselves in a manner which often resembles the regroupings described in our cases 2 and 3. It is of considerable interest, therefore, to inquire in what measure such sorting out, with its anatomically precise consequences, may be explained by the thermodynamic considerations which have been outlined. Precise measurement of the work of cohesion between living cells does not as yet appear to be feasible. It has proved possible, however, to examine in some detail the behavior of mixed populations of embryonic cells and to compare the observed behavior with that to be expected on thermodynamic grounds from a system conforming with one or another set of interunit adhesive relationships.

Chick-embryo heart and neural retinal cells, when mixed in appropriate proportions and allowed to coaggregate in a culture vessel, sort out to form islands of heart tissue totally encased by retinal tissue (27, 28) (Fig. 3). The system in this respect resembles that in our case 2, brought about by conformance with the adhesive relationships given in relation 4. The heart cells correspond with *a,* and the retinal cells with *b.* If these adhesive relationships actually operate to bring about the conformation depicted in Fig. 3, several predictions from thermodynamic theory ought to be fulfilled by the behavior of appropriate mixtures of such cells.

Prediction 1. The replacement of heart cells by retinal cells in the surfaces of heart-retina aggregates in which the heart cells are numerous should occur very early in the sorting out process, since reduction of surface free energy by this means requires far less rearrangement than a commensurate reduction of the total free energy of the system.

It was found that within 17 hours after the onset of aggregation, when sorting out was just beginning to be discernible, a marked depletion of heart cells was evident in the surfaces of the aggregates (27).

Prediction 2. When *a* units (heart cells) are very sparse in the population, so that their meeting one another is virtually precluded, minimization of surface free energy should cause them to be relegated to subsurface locations within the aggregates. They should be equally stable in all subsurface positions.
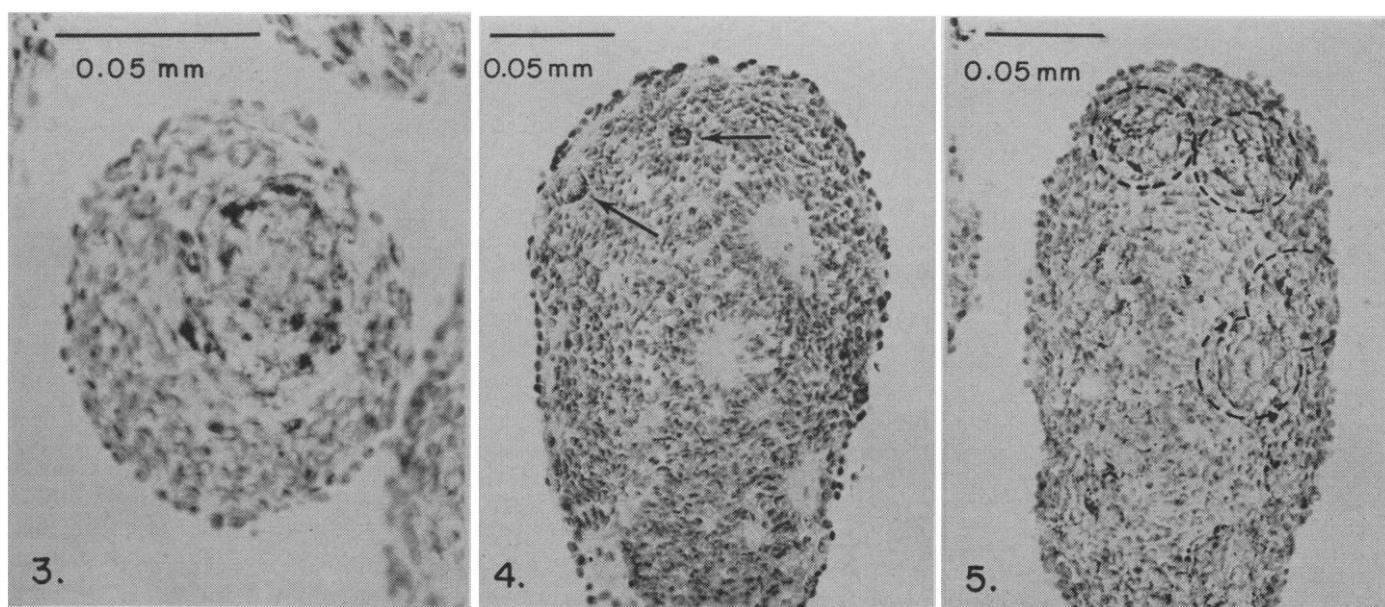

Fig. 3. Section through an aggregate formed by dissociated 5-day heart cells and 7-day retinal cells of chick embryo. Through sorting out, the reconstructed heart tissue has come to be enveloped by the reconstructed retinal tissue. Fig. 4. Section through an aggregate containing 99 percent retinal cells and 1 percent heart cells. The sparse heart cells, two of which are present in this section, leave the surface but otherwise remain generally distributed. Fig. 5. Section through a heart-retina aggregate early in the process of sorting out. Many small, discrete islets of heart cells (with black inclusions) have formed and are in the process of coalescing. Several islets are encircled on the figure.
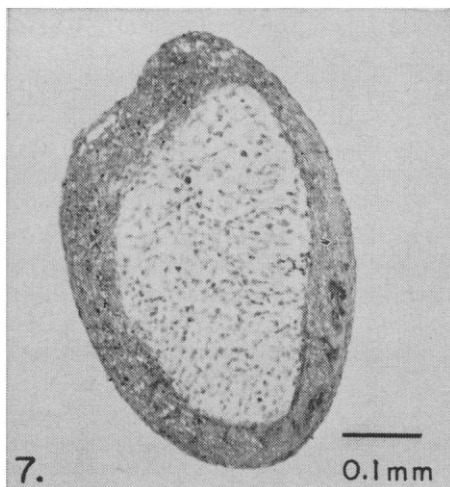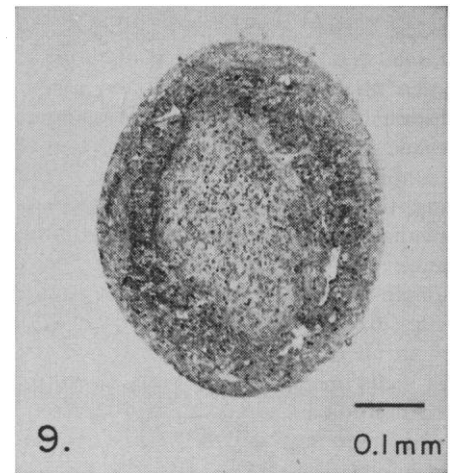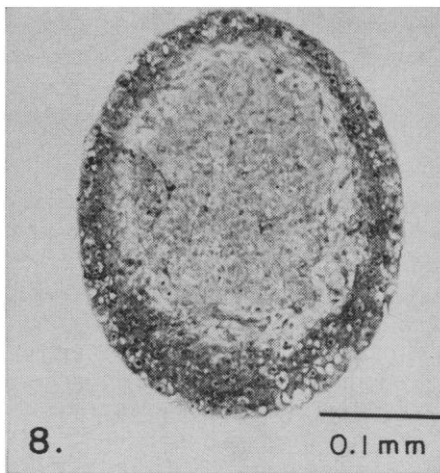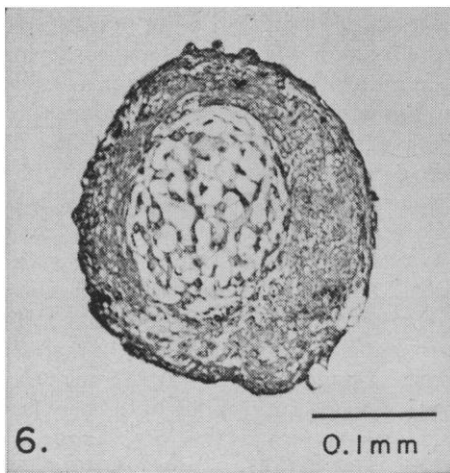
Fig. 6. Section through an aggregate formed by dissociated 4-day limb-bud chondrogenic cells and 5-day heart ventricle cells of chick embryo. The reconstructed heart tissue envelops the now-differentiated cartilage.

Fig. 7. Section through a structure formed by an intact fragment of the chondrogenic zone of a 4-day limb bud laterally fused with a fragment of 5-day heart ventricle. The heart tissue had spread over and enveloped the chondrogenic tissue prior to the deposition of matrix by the latter.

Fig. 8. Section through an aggregate formed by dissociated 5-day heart ventricle cells and 5-day liver cells. The reconstructed liver tissue envelops the reconstructed heart tissue.

Fig. 9. Section through an aggregate formed by dissociated 4-day limb bud chondrogenic cells and 5-day liver cells. The reconstructed liver tissue envelops the chrondrogenic tissue, in which the deposition of matrix has recently begun.

It was experimentally established (28) that sorting out of the two types of cells, to yield configurations such as that shown in Fig. 3, normally was accomplished within 2½ days. Reduction of the proportion of heart cells to 1 percent (by volume) of the population yielded aggregates whose surfaces at the end of this time were virtually devoid of heart cells, the latter being otherwise distributed apparently at random within the aggregates (Fig. 4). This result, in showing that heart cells do not "seek the center," would appear to exclude the possibility that directed migration plays a role in the sorting out of these cells.

Prediction 3. Sorting out should proceed by way of the progressive exchange of heteronomic adhesions for homonomic ones, in the course of which process the potentially internal tissue should appear as a discontinuous phase (that is, as coalescing islets), while the potentially external tissue should constitute a continuous phase.

Histological analysis of heart-retina aggregates fixed after graded intervals in culture bore out prediction 3 (27) (see Fig. 5). Similar observations have been reported for the sorting out of

mixed amphibian neurula chordamesoderm and endoderm cells (11) and of mixed pigmented retinal and wing bud cells from chick embryos (29).

Prediction 4. If the distribution of the two phases after segregation is that at which the system is in thermodynamic equilibrium, this same terminal distribution should be approached regardless of the initial distribution of the phases. Thus, lateral fusion of an intact fragment of tissue *b* with an intact fragment of tissue *a* should be followed by the progressive spreading of the one over the surface of the other to yield the same configuration which is ultimately produced through the sorting out of intermixed *a* and *b* cells.

The accuracy of this prediction has been established, to date, for 11 different combinations of tissue fragments and of their dissociated cells (30, 31). In each case, fusion of undissociated fragments of two tissues leads to the progressive envelopment of one fragment by the other, the final disposition of the two tissues being the same as that which is arrived at when the starting material is a mixed suspension of the corresponding dissociated cells. Of these 11 combinations, nine behaved in the manner described for our case 2

(Figs. 6 and 7), while two behaved in the manner described for our case 3. The latter showed partial retraction of the earlier continuous, external tissue after segregation within mixed aggregates; correspondingly, they showed only partial enclosure of one fragment by the other after fusion of intact fragments which had never been dissociated.

Prediction 5. In a segregating community composed of two kinds of mutually adhesive, motile units, the less cohesive phase will tend to envelop, partially or completely, the more cohesive phase at thermodynamic equilibrium. The motile cells of a series of different embryonic tissues constitute a series of phases, each of which is adherent to, yet segregates from, any of the others. Therefore, when the cell populations comprising such a series are intermixed in all possible binary combinations, the mutual positions which they come to assume at equilibrium should establish a hierarchy definable by the specification that if *a* is covered by *b* and *b* is covered by *c*, *a* will be covered by *c*.

In testing this prediction all possible binary combinations among cell suspensions derived from six different chick-embryo tissues have been used. There are, in all, 15 different combina-

406

tions. The segregation patterns obtained do indeed define a hierarchy such that one tissue is reconstituted internally in all combinations, another tissue is reconstituted externally in all combinations, and each of the remaining tissues falls into a specific intermediate ranking in complete accordance with prediction 5 (*31*). An example of this behavior is illustrated in Figs. 6, 8, and 9.

In all respects, then, the regroupings of cells in the populations which we have studied proceed along satisfyingly consistent and simple lines. They are precisely what is to be expected on thermodynamic grounds in any system composed of mobile units which are mutually adhesive, and between which certain quantitative adhesive relationships exist.

Those of us who have been seeking an explanation for sorting out and tissue reconstruction by dissociated cells have almost unanimously considered it necessary to assume that unlike cells adhere to one another less tenaciously than like cells do (expressed by relation 5)—a situation requiring selectivity in the mechanism of adhesion. The thermodynamic analysis of the situation shows, however, that under the circumstances of relation 5, while sorting out would be expected to occur, the reconstructed tissues would, in addition, be expected to continue their mutual self-isolation to a point at which each would come to occupy a portion of the surface of the aggregate (our case 3). Furthermore, the analysis shows that the most common outcome of segregation in a binary system—the production of a totally internal tissue entirely enveloped by another tissue—fulfills the expectation based upon a set of adhesive relationships quite different from that which we have previously assumed. In this set of relationships (relation 4), the unlike cells adhere to one another with a strength intermediate between the strengths of cohesion of the two kinds of like cells. Does logic lead us, then, to postulate the existence of selectivity in the adhesion mechanism itself?

The simplest possible assumption capable of accounting for type-specific differences in the strengths of adhesions between cells is the assumption that adhesive sites of a single kind are scattered more abundantly on the surface of one type of cell than on the surface of another. W, the work of adhesion between two cells, would then be directly proportional to the number of

adhesive sites which are apposed, per unit of area, at the junction between the two cells. What would be the adhesive relationships which would derive from the operation of this simplest of systems?

If the frequency of adhesive sites per unit area on the surfaces of cells a and b is designated f_a and f_b, respectively, the probability of apposition of sites in the cell pairs *a-a*, *b-b*, and *a-b* is given by $(f_a)^2$, $(f_b)^2$, and $(f_a)(f_b)$ for the respective cases. Introducing the proportionality constant k, we may write the equations

$$W_a = k\,(f_a)^2 \qquad (7)$$

$$W_b = k\,(f_b)^2 \qquad (8)$$

$$W_{ab} = k\,(f_a)(f_b) \qquad (9)$$

Following the convention that $W_a \geqq W_b$, we obtain

$$f_a \geqq f_b \qquad (10)$$

Multiplying both sides of relation 10 by the value $f_a \cdot f_b$ and rearranging, we obtain

$$\frac{(f_a)^2 + (f_b)^2}{2} \geqq (f_a)(f_b) \qquad (11)$$

Multiplying both sides of relation 10 by the value f_b, we obtain

$$(f_a)(f_b) \geqq (f_b)^2 \qquad (12)$$

Combining relations 11 and 12, we get

$$\frac{(f_a)^2 + (f_b)^2}{2} \geqq (f_a)(f_b) \geqq (f_b)^2 \qquad (13)$$

Substituting Eqs. 7–9 in relation 13, we obtain

$$\frac{W_a + W_b}{2} \geqq W_{ab} \geqq W_b \qquad (14)$$

Relation 14, representing the adhesive relationships which would be engendered in this simplest of systems, will be recognized as an expression of the limits represented by relation 4. And the conditions expressed by relation 4 are precisely those which yield, at thermodynamic equilibrium, our case 2—the result most commonly obtained experimentally, in which one phase is totally enveloped by the other. It is not necessary to assume the literal existence of discrete adhesive sites as distinguished from nonadhesive sites among which they are distributed. Relation 14 applies to any case in which the force between two mutually adhesive (attractive) bodies is proportional to the product of the individual adhe-

sive (attractive) forces. It may be seen from Eqs. 7–9 that the values of W_{ab} generated in this system are given by

$$W_{ab} = \sqrt{\overline{W_a}} \cdot \sqrt{\overline{W_b}} \qquad (15)$$

These values are represented by the dotted line in Fig. 2.

This analysis shows, then, that (i) the mutual sorting out of two kinds of cells to reconstitute tissues, one of which encloses the other, and (ii) the spreading of an intact fragment of the one tissue to envelop an intact fragment of the other are precisely the phenomena which are to be expected, in accordance with the principle of minimization of free energy, in the total absence of selectivity in the adhesion mechanism itself. Only quantitative differences in adhesiveness are necessary. The "information" required in the adhesion mechanism is, in such cases, restricted to "more" and "less."

This does not mean, of course, that molecules of different sorts, on the surfaces either of cells of a given kind or of cells of differing kinds, may not in such cases participate directly in the mediation of adhesions. It merely means that whatever the chemical nature of, or diversity among, the adhesives themselves, the quantitative adhesive relationships among the cells which bear them would be expected to approximate, within the limits shown in Fig. 2 (see also *24*), the relationships derived from the simple postulates which have been outlined. In cases in which, at equilibrium, one tissue covers the other incompletely or not at all, it becomes necessary to assume the additional operation of some other factor or factors, such as an ordered distribution of, or qualitative nonidentity among, adhesive sites.

Morphogenesis and Specificity

Thus we return, at the end, to the beginning: Where is the common denominator? What has sorting out to do with normal morphogenesis? Sorting out, after all, is not known to play a major role in morphogenesis. Such a role, however, *is* played by *spreading:* the spreading of one tissue over the surface of another, or—what is the equivalent—the penetration of one tissue into a mass of another. Differences in cellular adhesiveness which may be built into a system of tissues to bring about the spreading of one tissue over another, or the penetration of one tissue into another, would incidentally

(and coincidentally) provide all the conditions required, in an artificial mixture of cells, for sorting out to occur, and for its morphological result to imitate the anatomy normally produced by mass tissue movements. The foundation for such a thermodynamic analysis, like much of the empirical groundwork upon which it rests, was laid by Holtfreter (32), whose treatment of the subject has been discussed separately (33).

Our recognition of the organization which is everywhere present in the living world has played a prominent role in the development of our biological concepts. It is not surprising that apparent meaningfulness or complexity in the design and functioning of organisms should have led us to assign corresponding attributes to the mechanisms governing the functioning and the design. Yet, as knowledge has grown, complex explanations have had a way of succumbing to relatively simpler ones. Thus, overt vitalism is gone from the scene. Organic molecules, it later developed, could be synthesized by the chemist after all. Proteins were not so simple as to preclude the possibility of their functioning as enzymes; nor was DNA, at a later stage, too simple to provide the vast stores of "information" for which the proteins, now recognized to be complex, might have seemed a more fitting receptacle.

While the *adaptedness* brought about through evolution appears complex, the *adaptiveness* which makes evolution possible is born of simplicity. The entire genetic code (and more) is expressible with an alphabet containing only four elements. It would appear that a not inconsiderable amount of the "information" required to produce, through morphogenetic movement, the anatomy of a body part may be expressed in a code whose sole element is quantity: more versus less. There is, I think, reason to expect that as more realms of biological specificity yield to analysis, their most impressive feature may be the simplicity of the terms in which specificity—information, if you will—can be expressed (34).

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Revolutions in Physics and Crises in Mathematics

There is no simple, single formula for the course of revolutions in science.

Salomon Bochner

In this article I deal with two topics which, although separable, are closely connected with each other. The first and larger part of the article is concerned with the conception of a revolution in physics, as recently blue-printed in a provocative book by Thomas Kuhn (1). I make observations which are seemingly in conflict with those of Kuhn, but I really intend to amplify and qualify some of Kuhn's theses rather than to dissent from them, and my approach is somewhat different anyhow. After that, I make some observations on revolutions in physics as far as the underlying mathematics is concerned. And, finally, I make some remarks on so-called "foundation crises" in mathematics, which may be viewed as a kind of revolution, and especially on a major crisis of this kind which is presumed to have taken place in the 5th century B.C.

Kuhn, in his investigations into the nature of revolutions in science, analyzes both the inward ontological and epistemological nature of such revolutions and the psychological and behaviorist attitudes, resistances, and responses of practitioners of science, before, during, and after a revolution. Kuhn finds that revolutions in science are mostly internal revolutions, brought about by some scientists and then forced by the initiators on the scientific community at large. There is even an implied suggestion that, in the begin-

408