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Strategies for Specifying Form and Pattern: Adhesion-Guided Multicellular Assembly

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Strategies for specifying form and pattern: adhesion-guided multicellular assembly

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[Plates 1 and 2]

We define a material pattern as a particular arrangement of material elements in space. We then make an effort to categorize the developmental strategies that underlie the emergence of multicellular patterns. These strategies are divided into three broad categories according to whether cell position influences or is influenced by cell fate. In that category of strategies in which cell fate influences cells to move to particular positions, we focus our attention upon morphogenetic and patterning phenomena that appear to be determined by adhesion-mediated interactions of cells with each other and with their surroundings. The differential adhesion hypothesis details how cellular adhesive properties can guide tissue movements and specify patterns of cell association. Motile, adhesive cells will naturally tend to group so as to maximize their adhesive interactions (minimize interfacial free energy). A homogeneous population of uniformly adhesive (isotropic) cells will tend toward spherical form. Cell surface adhesive anisotropies can determine other most-stable (equilibrium) configurations of the population, such as cell sheets, tubes and vesicles. Heterogeneous cell populations may preferentially either intermix or sort out, depending upon the balance of adhesive forces between like and unlike elements. The precise configuration adopted will depend upon the particular adhesive relationships that prevail. Both this end state and the approach toward it arise from the adhesive relationships among the interacting cells. Such morphogenetic phenomena as tissue spreading and the segregation of organ primordia are probably brought about in this way. We outline here some results of our recent experiments on the morphogenesis of the salamander pronephric duct. These illustrate the reality of emergent adhesion-generated tissue immiscibility as a cause of organ segregation and point toward a craniocaudally travelling adhesion gradient as the information that guides the migrating pronephric duct to the cloaca.

1. Alternative strategies for the specification of form and pattern

Tissues, organs and organisms consist of cells and cell products arranged in specific shapes and patterns. It is our purpose here to consider the general strategies that multicellular systems use to order themselves; to make an attempt at classifying these strategies; to review some of the morphogenetic phenomena that intercellular *adhesive* interactions can bring about; and to give some evidence supporting the operation of adhesive control mechanisms in the governing of morphogenesis and pattern formation in developing embryos.

A material pattern can be defined as a particular arrangement of material elements in space. Many of the biological patterns that concern us here involve particular arrangements of cells

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of different kinds. Specification of such a pattern requires specification of the *identity* and the *position* of each element in the pattern. Strategies for producing multicellular patterns, classified according to the means by which these two parameters are determined and coordinated, then fall into three broad categories (see table 1).

TABLE 1. STRATEGIES FOR MULTICELLULAR PATTERNING

strategy	*related concepts and processes*
I. position influences cell fate	dependent differentiation, induction, regulation, positional information
(A) specification of existing cells	morphallaxis
(B) production and specification of new cells	blastogenesis, intercalary regeneration, epimorphosis
II. cell position neither influences nor is influenced by cell fate	mosaicism, predetermination
III. cell fate influences position	morphogenetic movement
(A) cells change shape but retain their neighbours	epithelial thickening, thinning, bending and folding
(B) cells change their neighbours	cell and tissue migration
(1) in response to signals from a distance	(chemo)taxis
(2) in response to local, contact interactions	
(*a*) due to 'signalling'	'contact inhibition type 1' (Vesely & Weiss 1973)
(*b*) due to adhesive interactions	'contact inhibition type 2' (Vesely & Weiss 1973), haptotaxis, tissue spreading, cell sorting, delamination, multicellular assembly, segregation of organ primordia

(*a*) *Position influences cell fate*

In the first of these categories, pluripotential cells are induced by environmental variables to *differentiate* in a manner appropriate to their positions. L. Wolpert has called the system of environmental cues that elicit this differentiation 'positional information' and wishes to restrict the application of the term 'pattern formation' to cases in which this strategy alone is employed. We do not see any justification for such a restriction. The responding cells may pre-exist ('morphallaxis'; Morgan 1901); or may be produced in response to the need for them ('epimorphosis'; Morgan 1901).

(*b*) *Cell position neither influences nor is influenced by cell fate*

In the second category, cells prespecified at birth are produced in essentially their definitive positions with respect to their neighbours. Fate and position are individually but quite stringently specified and altering one does not alter the other. So-called mosaic systems belong here.

(*c*) *Cell fate influences position*

In the third category, cells already possessing differentiated properties are caused to *move* to positions appropriate for them. Three substrategies can be recognized here. In the first of these, cells maintain their mutual positions but change their shapes, either elongating or constricting at one end. These shape changes of anchored cells summate to produce stretching, shrinkage or curvature of the cell sheet. In the second case, cells are guided to their destinations by signals coming from a distance (taxis). In the third case, cells are guided to their destinations by local, contact interactions with each other or with their surroundings. In this last category are the adhesion-mediated interactions upon which we shall now focus.

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2. Adhesion-mediated cell and tissue guidance

In classifying these behaviours, it is necessary to consider the general adhesive properties of both the cell substratum, if one is present, and of the cells themselves.

(*a*) *Substratum adhesive properties*

For simplicity, we consider here only two-dimensional substrata, although three-dimensional substrata (e.g. thick gels that admit cells) also exist and play an important role in embryonic morphogenesis. The categories of morphogenetic phenomena that are here related to properties of two-dimensional substrata can readily be extrapolated to three-dimensional substrata as well.

(i) *Uniform substratum*

In this case, the substratum is isotropic and uniform in its adhesive interactions with cells. It cannot, therefore, guide cells in any particular direction but acts only as a surface upon which cells move. This does not mean, however, that the movement of cell populations over such a substratum must lack consistent direction. Cells commonly compete for space upon such a substratum and, if the cells regularly originate in a specific region, this competition easily leads to spreading of the cell population in a specific direction. According to present views, the cell competition ('contact inhibition' in its original sense (Abercrombie & Heaysman 1954)) can be of two kinds. A cell may, upon contact with another cell, experience a direct inhibition of its *locomotion* toward the cell which it has contacted; or cells may tend to avoid mutual *overlapping*. The latter circumstance could arise from differences in the intensities of cell–cell and cell–substratum adhesions (Abercrombie 1961; Garrod & Steinberg 1973, 1975; Harris 1973; Martz & Steinberg 1974; Martz *et al.* 1974; Steinberg & Garrod 1975). Whatever the precise mechanism of contact inhibition in any specific instance, as long as the cells tend to monolayer and increase in number, the population as a whole will expand toward unfilled space. Behaviour of this kind is seen not only *in vitro* (reviewed in Heaysman 1978) but also *in vivo* in such morphogenetic movements as the spreading of embryonic mesoderm between ectoderm and endoderm during gastrulation, in the spreading of the vertebrate embryonic epicardium over the myocardium (Ho & Shimada 1978) and in the spreading of the avian corneal endothelium between the corneal stroma and the lens capsule (Bard *et al.* 1975; Nelson & Revel 1975). Other sets of adhesive relationships or failure of locomotory inhibition (Abercrombie *et al.* 1957; Martz *et al.* 1974) can lead to multilayering of cells.

(ii) *Oriented substratum*

Here cells attach to and move along structures in the substratum. These structures orient cell movement but permit it to proceed equally well in either direction. This has been called 'contact guidance' by Weiss (1947). Oriented substrata could lead to oriented final configurations of cell populations as well as to oriented cell locomotion. Actually the distinction between a uniform and an oriented substratum may sometimes be a matter of scale. If the substratum is isotropic but narrow and long, it might be regarded as oriented. The developing vertebrate nervous system offers a number of examples (Weiss 1955; Ramón y Cajal 1960; Rakic 1971; Ebendal 1976, 1977; Dunn & Ebendal 1978; Singer *et al.* 1979; Silver & Sidman 1980).

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(iii) *Polarized substratum*

In this case the substratum possesses not only orientation but polarity as well, causing cells to move preferentially in a single direction. Cell movement up a gradient of increasing adhesiveness to the substratum, called 'haptotaxis' (Carter 1965), provides a case in point. This could cause cells to accumulate at regions of high substratum adhesiveness. It has been suggested that the movement of sea urchin primary mesenchyme cells (Gustafson & Wolpert 1961) and of the chick embryonic myocardial primordia (deHaan 1964) may be guided in this manner. Our own recent experiments, some of which are discussed below, provide evidence for the operation of such a mechanism in the guidance of the migrating urodele pronephric duct. Similar migratory behaviour could be brought about if a uniform substratum materialized in a specific temporo-spatial sequence. This could produce polarized spreading of cells or extension of neurites due to a *temporal* rather than a spatial polarity of the substratum.

(*b*) *Cell adhesive properties*

(i) *Uniform cell adhesiveness*

One can question whether animal cells are ever truly 'isotropic' (Goel *et al.* 1970) or uniform in adhesive properties over their entire surfaces, but for convenience and simplicity they have often been modelled as though they were (e.g. by Steinberg 1963, 1964; Goel *et al.* 1970; Martz *et al.* 1974).

(ii) *Non-uniform cell adhesiveness*

Cells commonly have 'anisotropic' (Goel & Leith 1970) adhesive properties. In a simple case, the anisotropy may consist of adhesive patches separated by cell surface expanses of lower adhesiveness (Revel *et al.* 1974; Revel & Wolken 1973; Izzard & Lochner 1976). In a more complex form of anisotropy, a cell may have various domains of its surface specialized for adhesion to different structures (e.g. basement membrane or particular regions of other cells' surfaces) or lacking in adhesiveness, as in membrane areas lining cavities or on the outside of the body. Intercellular junctions such as tight junctions, belt and spot desmosomes and gap junctions are mirror-symmetric organelles shared by apposed cell surfaces and participating in intercellular adhesion. Their structural specialization indicates that they are chemically specialized as well. The fact that mismatched junctions (e.g. a half-desmosome apposed to a half-gap junction) do not occur means that the adhesion-mediating constituents of each kind of junction have *junctional specificity*, which is to say that they form stable associations only with adhesion-mediating components of junctions of like kind. Whether the same kind of recognitive heterogeneity exists also in the structurally undifferentiated regions of intercellular adhesion is not yet known. But it seems certain that each kind of cell must possess at least as many different kinds of adhesion system as it possesses ultrastructurally unique kinds of association structure. Since each kind of junction or association structure on the cells of an organized tissue is found in a particular cell surface location, it follows that their pattern reflects a topographic mosaic of differentiated cell surface adhesion properties.

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3. Adhesive specification of structure and pattern

Motile cells will tend to adopt those positions and orientations in which they are held most firmly. This idea is in itself very easy to accept. But, when it is pursued in quantitative detail, some of its consequences have proved to be far-reaching and, to some, unexpected and even counter-intuitive. The behaviour expressed by any population of mobile, mutually adhesive elements in the course of maximizing adhesion is characterized as liquid. In the *differential*

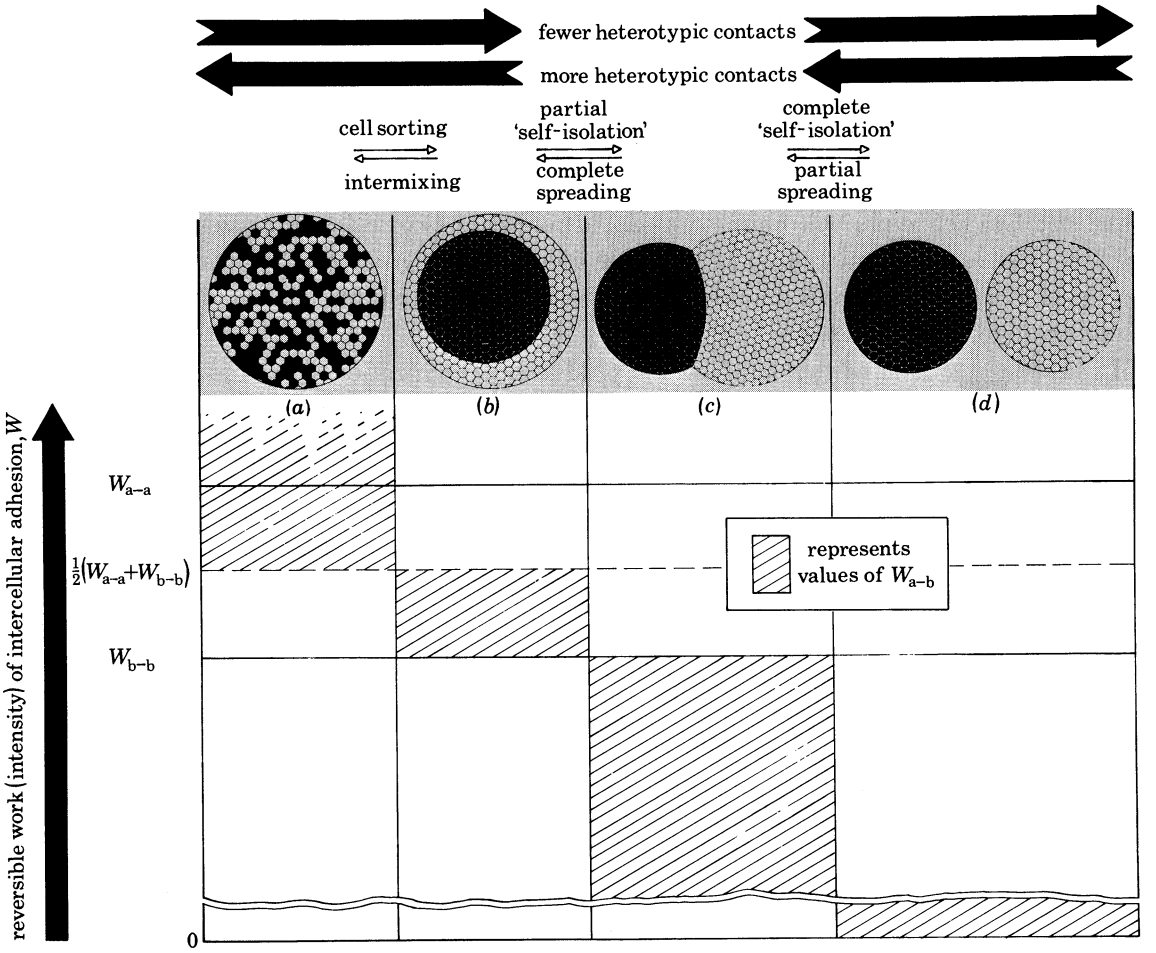

FIGURE 1. Illustration of how the reversible works of cohesion (W_{a-a} and W_{b-b}) and adhesion (W_{a-b}) determine the most stable configuration of a liquid system. These relationships should apply to any multi-subunit system that adopts liquid-like equilibrium shapes, whether the subunits are molecules or cells. (Reprinted from Steinberg (1978*b*).

adhesion hypothesis (Steinberg 1962, 1963, 1964, 1970, 1978*a*, *b*), we advanced the proposal that the liquid-like properties of multicellular aggregates result from the maximization of adhesions (minimization of interfacial free energy) and laid out the specific, quantitative adhesive relationships between and among cells of different kinds that would specify a number of most-stable or equilibrium configurations of the global cell system. For the sake of simplicity, we limited our detailed analysis to systems containing no more than three types of cells of uniform adhesiveness, pointing out, however, that 'regional surface differentiations may function to bring about and to establish rather complex constellations of tissues' (Steinberg 1964).

It is our purpose in this section to summarize the kinds of morphogenetic and patterning phenomena that can result from the expression of cellular adhesive properties.

(*a*) *Morphostatics: tissue configurations and histological patterns*

The equilibrium configurations corresponding to all possible sets of relative adhesive intensities between uniformly adhesive cells of two kinds are shown diagrammatically in figure 1. Maximization of intercellular adhesions produces tension in the tissue surface and a consequent tendency toward the adoption of a smoothly curved, spherical form. If the intensity of heterotypic adhesions (1) equals or (2) exceeds the mean of the intensities of the corresponding homotypic adhesions, the two cell types will intermix randomly in the former case and preferentially in the latter (figure 1*a*). Cell surface adhesive anisotropies have the potential for bringing about much more complex histological patterns, as has already been noted.

If the intensity of heterotypic adhesions is below the mean of the homotypic adhesive intensities, the two cell types will be most stably arranged as two separate masses or phases, with the less cohesive tissue surrounding the more cohesive to a greater or lesser degree. Even the degree of this envelopment is determined by the set of relative intercellular adhesive intensities (Steinberg 1962) (figure 1*b–d*).

It is characteristic of this strategy for producing organization that the totality of a cell system's adhesive energies specifies a particular pattern or configuration as most stable. A system structured according to this principle will be self-stabilizing, responding to any perturbation by restoring itself to its original organization. This provides a simple explanation for the form-regulating property of so many multicellular structures.

(*b*) *Morphodynamics: the assumption of new forms and patterns*

Just as a particular set of adhesive properties can stabilize an existing structure against alteration, so can it also act to bring this structure about through cell rearrangements when the system starts out in some other configuration. We have recently discussed these rearrangements (Steinberg & Poole 1982) and merely categorize them here.

1. *Rounding-up* of a tissue has already been mentioned; as a consequence of maximizing the area of intercellular adhesions, the exposed, adhesive surface area is minimized. The body of minimal surface area is, of course, a sphere. We do not all become spheres, however, because our bodies possess skeletons and other solid scaffoldings; because not all cells remain mobile; and because not all cell surfaces are adhesive. If the non-adhesive surface area of a cell system exceeds the area of the surface of a sphere of the cell system's volume, the system will be most stable in a non-spherical form.

2. *Cell infiltration* could be caused by the coming-together of two cell populations whose cells cross-adhere with sufficient strength (figure 1*a*).

3. *Spreading of one cell population over another* will occur when the former adheres to but is less

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Description of plate 1

Figure 2. Scanning electron micrograph of stage 24 axolotl embryo fixed before peeling ectoderm from right side. Arrow indicates pronephric duct's caudal tip.

Figure 3. A pronephric rudiment and some adjacent somite and lateral mesoderm from a stage 22 axolotl donor were transplanted ventral to the pronephric rudiment of a stage 22 host. The secondary duct rudiment has extended across the host flank by the dorsocaudal migration of its caudal tip and has fused with the host's pronephric duct. The epidermis was peeled off after fixation.

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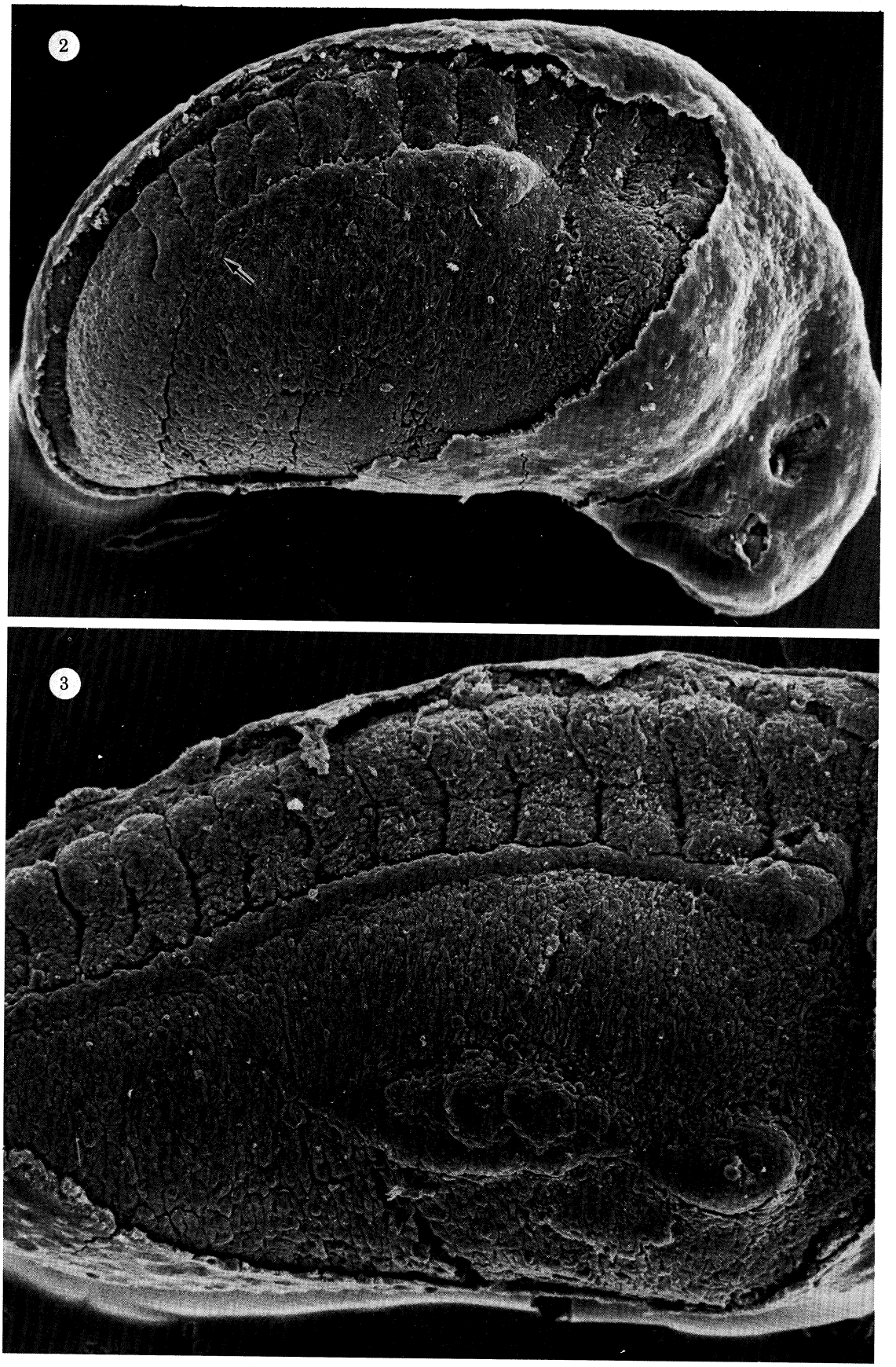

FIGURES 2 AND 3. For description see opposite.

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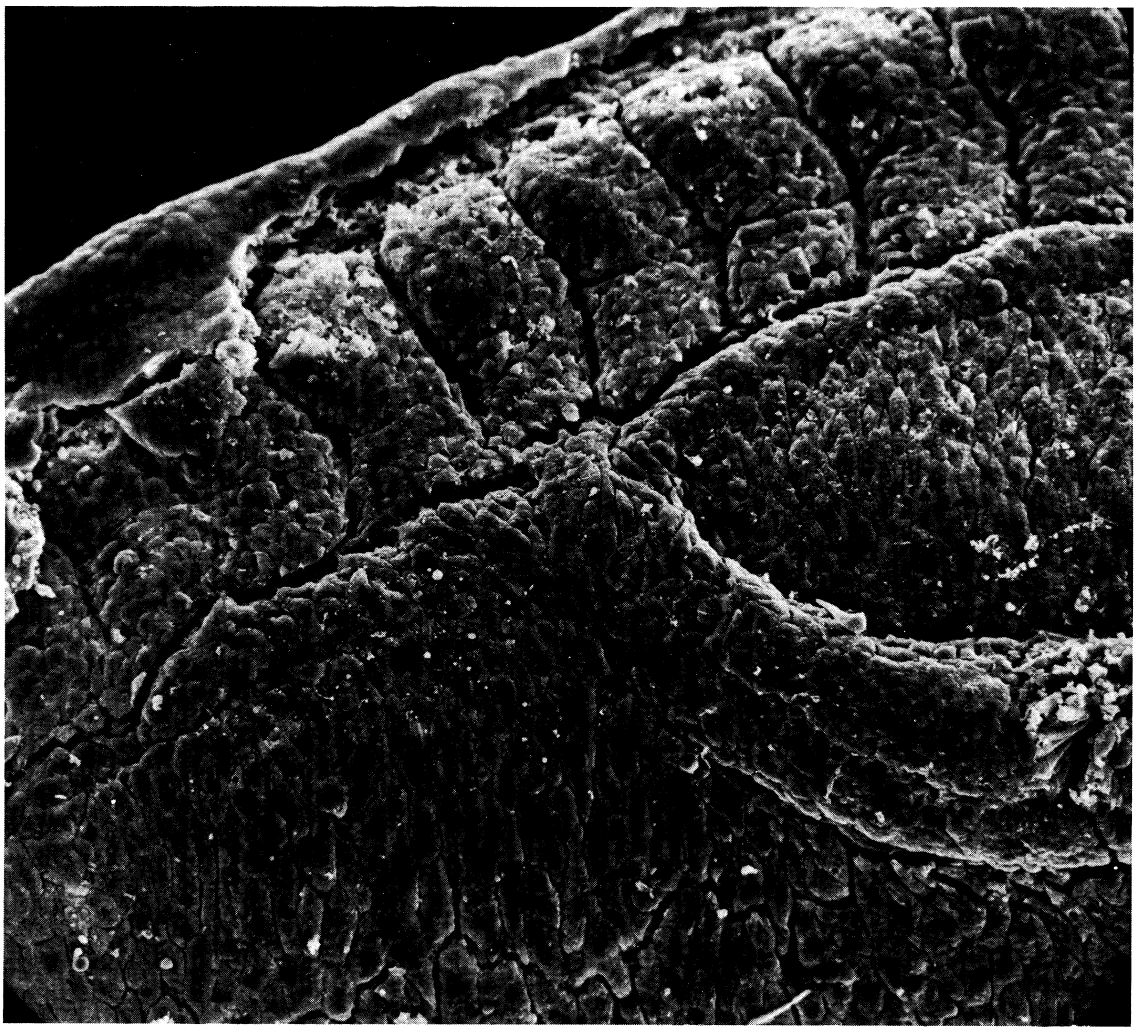

FIGURE 4. After this operation, similar to that shown in figure 3, the grafted somite mesoderm (above) streamed dorsocaudad along with the migrating pronephric duct. Upon encountering the boundary between somites and primary pronephric duct, the co-migrating streams fused, duct with duct and somite mesoderm with somite mesoderm, demonstrating the miscibility of like tissues and the immiscibility of unlike tissues.

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cohesive than the latter (figure 1*b*, *c*). If the substratum is oriented, the spreading may likewise be oriented. If the substratum is polarized (e.g. if it displays a gradient of adhesiveness), a cell or tissue spreading upon it may move in a specific direction and may vacate space previously occupied. The salamander pronephric duct presents a particularly interesting variation on this theme (Poole & Steinberg 1981; Steinberg & Poole 1982), briefly summarized below.

4. *Sorting-out* of originally intermixed cells is the opposite of cell infiltration and would result if the intermixed cells cross-adhere with *insufficient* strengths (figure 1*b–d*).

5. *Segregation of organ primordia* deserves more attention than it has received as a probable example of a normally occurring, adhesion-mediated embryonic phenomenon (Heintzelman *et al.* 1978; Steinberg & Poole 1982). Proof is of course required in each specific instance, but such phenomena as the delamination of the hypoblast in avian and mammalian embryos and the segregating-out of notochord, somites, pronephric rudiment and lateral mesoderm from the originally smooth mesodermal mantle are easily explained as due to the acquisition of new adhesive properties, which render each domain immiscible with its neighbours. There are elements of both morphogenesis and pattern formation here, for as pointed out earlier by Waddington (1956, p. 415), '...we should remind ourselves that regionalization normally takes place so as to produce definite patterns of arrangement of the different parts. It is not adequate to picture it merely as a process by which a number of intermingled entities become sorted out into heaps of like components; we must add the fact that the heaps are mutually arranged in orderly patterns.'

The process called *compartmentalization* and demonstrated particularly in insect systems may be closely related to what Waddington calls regionalization. Nübler-Jung (1977) has provided strong evidence that intercellular adhesive differentials are involved in the stabilization of those compartments represented by the abdominal segments of the bug *Dysdercus intermedius*, and Nardi & Kafatos (1976; Nardi 1977) provide evidence for a similar system operating in the morphogenesis of the insect wing.

4. Adhesion-guided assembly in the embryo: morphogenesis of the pronephric duct

For some time we have been engaged in a study of the morphogenesis of the pronephric duct in various vertebrate species. We selected this organ because of its accessibility to both experimental manipulation and scanning electron microscopic observation and because its mode of development suggested it as an ideal system for investigation of the mechanisms underlying a directed tissue migration. Figure 2, plate 1, depicts the exposed flank mesoderm of an *Ambystoma mexicanum* (axolotl) embryo at Schreckenberg & Jacobson (1975) stage 24. The pronephric rudiment has segregated from the rest of the mesoderm between trunk somites 2 and 7 as an ovoid mass. Its posterior end is here in the process of migrating caudad along the ventrolateral border of the somites, stretching the duct rudiment out between the head of the pronephros, which anchors the duct anteriorly, and the cloaca, with which the migrating duct tip ultimately fuses (Poole & Steinberg 1977, 1981). A change in the cohesiveness of the emerging pronephric rudiment is suggested by observations that it bulges outwards and its cells appear more compacted than those of the subjacent lateral mesoderm, from which the duct rudiment becomes demarcated by the appearance of a boundary similar to those that appear between newly segregating somites.

It was our aim to identify the general nature of the mechanism that guides the migrating duct tip along its precise path to the cloaca. We approached this question through consideration of the possible guidance mechanisms available and the behavioural modalities associated with each. These have been classified in §2 above and are outlined in table 2.

Pronephric duct rudiments transplanted to the flank of a host embryo below the primary duct consistently migrated in a dorsocaudal direction across the flank mesoderm to fuse with the primary duct (figure 3, plate 1). Migration was never in either a ventral or an anterior

TABLE 2. CELL AND TISSUE GUIDANCE STRATEGIES AND THE MIGRATORY BEHAVIOUR THAT EACH WOULD ENGENDER

		expected behaviour	
source of guidance information	substratum properties	migration persists after removing distant tissue	migration path
distant		−	unidirectional
local	uniform	+	omnidirectional
	oriented	+	bidirectional
	polarized	+	unidirectional

direction regardless of the orientation of the graft. In some experiments the primary duct was experimentally blocked and the dorsocaudally migrating secondary duct then encountered the normal duct's empty path. In each case (five embryos) the grafted duct turned decisively caudad, even when its angle of approach to the host's duct path was close to 90°.

The unidirectionality of the duct's migration is consistent with either of two guidance mechanisms: taxis (e.g. chemotaxis) or movement on a polarized substratum (e.g. haptotaxis; migration up an adhesive gradient). To distinguish between these alternatives, we removed all the tissues that might possibly serve as distant sources of attraction. Removing the posterior fifth or fourth of the host embryo removes all tissues that might be attracting the primary duct. This includes the cloaca as well as the unsegmented somite mesoderm and caudal primary axial structures. This did not inhibit the migration of the primary duct, which in each case extended along the dorsal margin of the wound. Nor is a secondary duct attracted dorsocaudad by the primary duct, since restriction of the primary duct to the anterior part of the body by a dorsal cut did not alter the dorsocaudad extension of a transplanted duct rudiment. Thus no evidence could be found for chemotaxis or other distant-signalling guidance mechanisms. We have concluded that the migrating salamander pronephric duct is guided by a polarized substratum.

Our studies have revealed that the duct-guiding information is not static but moves caudad along the flank mesoderm as a wave precisely synchronized with the anteroposterior wave of somite segmentation (Poole & Steinberg 1982). Our evidence suggests that the duct-guiding information is a caudally moving gradient of flank mesoderm adhesiveness and that the duct tip is moving up this moving adhesion gradient in the manner shown for fibroblasts on a static gradient *in vitro* (Carter 1965; Harris 1973).

One last set of observations will serve to underscore the reality of the development of immiscibility relations between liquid tissues as a mechanism for causing tissue segregation. We observed that, on occasion, pieces of ventral somite mesoderm transplanted as part of our pronephric grafts would migrate alongside the streaming duct mesoderm. Two such streams migrate in lateral contact but never mix, the line of demarcation between them being distinct at all points. When these conjoined streams come in contact with the host paraxial mesoderm,

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the secondary and primary ducts fuse with no trace of a boundary. The stream of somitic mesoderm, however, demonstrates its immiscibility with the primary pronephric duct by spreading over it and fusing instead with the host's somite mesoderm (figure 4, plate 2).

These observations demonstrate the miscibility of duct with duct and of somite mesoderm with somite mesoderm, their immiscibility with each other, and the immiscibility of each with the lateral mesoderm. Before the duct mesoderm appears, of course, there is no sign of any boundary between prospective duct and lateral mesoderm. The same is true with respect to the as yet unsegmented somite mesoderm and the lateral mesoderm. Thus the emergence of these organ rudiments from the mesoderm mantle is accompanied by the appearance of immiscibility among all of them, in keeping with the idea that the development of mutual immiscibility, itself a reflection of changed intercellular adhesive properties, is the mechanism by which such organ primordia segregate out.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 2 AND 3. For description see opposite.

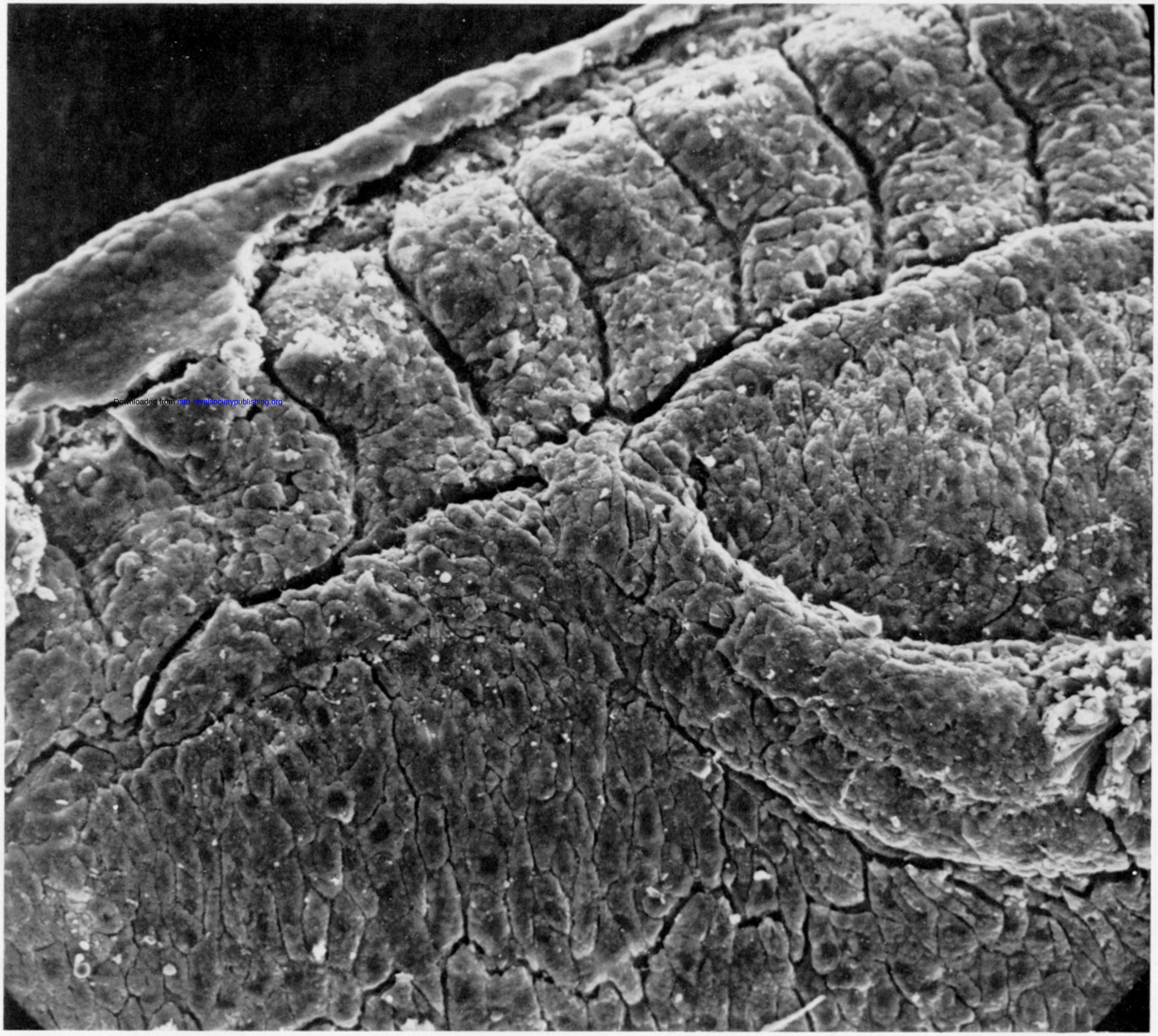

FIGURE 4. After this operation, similar to that shown in figure 3, the grafted somite mesoderm (above) streamed dorsocaudad along with the migrating pronephric duct. Upon encountering the boundary between somites and primary pronephric duct, the co-migrating streams fused, duct with duct and somite mesoderm with somite mesoderm, demonstrating the miscibility of like tissues and the immiscibility of unlike tissues.