

A Simple Model for Early Morphogenesis

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Despite the large amount of knowledge which continues to accumulate about early developmental events, very little is known about the processes which control them. Part of the problem may lie in that workers applying different approaches and techniques have different points of view and appear to be reluctant to read each others' literature. My aim in this paper is not to give a generative, formal model for early development, but rather to suggest several connecting strands between the physiological, biochemical, cell biological and experimental embryological approaches which may stimulate new research in fields *between* those already exploited.

Background

After cleavage stages in vertebrate embryos, during which the cells (blastomeres) of the embryo increase in number, two important processes take place: blastula formation and gastrulation. These two processes are crucial for the formation of the basic body plan of the organism. In general, the process of blastula formation consists of the formation of a cavity, the blastocoel, although the details of this process differ slightly in different species of vertebrates, depending on how much yolk they contain. By the end of blastula formation, vertebrate embryos usually consist of two main layers of cells (germ layers): an ectodermal layer (ectoderm or epiblast) dorsally and an endodermal layer (endoderm or hypoblast) ventrally. During the process of gastrulation in vertebrate embryos, visible bilateral symmetry is established by the formation of the first morphological axial structure. In birds and mammals this structure is known as the *primitive streak*. It consists initially of a thickening of a region of the ectodermal layer due to accumulation of cells. The streak serves as a passageway for cells leading to the formation of the third germ layer, or mesoderm, which becomes situated between the ectoderm (epiblast) and the endodermal layer. In rough terms, the ectoderm will eventually give rise to the skin and neural system, the endoderm to the digestive system and the mesoderm to the coelom, circulatory system and most of the internal organs of the organism.

Gastrulation is followed by neurulation, which consists of the early stages in the formation of the nervous system. Neural competence arises in the ectoderm from the interaction ("neural induction") between this layer and the underlying mesoderm. At about the same time, the primitive streak shortens in length, the notochord is laid down axially (this structure will give rise to part of the body of the vertebrae) and the somites segment from the mesoderm on either side of the axis. By this time the basic body plan of the embryo has been established.

Very little is known about the controls of these processes. In this paper I shall outline the principles of a simple conceptual framework in the light of which the phenomenology of early vertebrate development may be viewed and tentatively re-interpreted. I shall use the early chick embryo as a model system, as at these early stages of its development it has very simple geometry (a flat disc known as the "blastoderm"), and because much of the coarse phenomenology of these early stages is well documented in the literature (Vakaet, 1970; Nicolet, 1971; Hara, 1978; Stern & Ireland, 1981; Bellairs, 1982).

Antero-posterior polarity in the chick embryo is determined *in utero*, probably by the action of gravity during rotation of the egg (Kochav & Eyal-Giladi, 1971) but it does not become irreversibly established until the primitive streak forms, some 12 hours' incubation after laying. I shall mostly concern myself with the processes involved in the establishment of the primitive streak and with the maintenance of this polarity.

Jaffe & Stern (1979) have recently described the presence and spatial pattern of extracellular electrical current flow in early chick embryos at primitive streak stages. It was suggested that this current reflected transepithelial cation transport by a pump operating in the epiblast. This tissue appears to pump sodium ions into the underlying cavity and potassium out of it (Howard, 1957; Stern, 1982a; Stern & MacKenzie, 1983), whilst as in most transporting epithelia, water follows in the same direction as sodium (New, 1956; Elias, 1964). The pattern of extracellular current flow (Jaffe & Stern, 1979) closely follows the direction of migration of cells at the level of each of the germ layers, suggesting a causal relationship between the two phenomena (an idea which was already proposed by Cohen & Morrill, 1969). Nevertheless, I have recently shown (Stern, 1981) that any lateral voltage gradients which might be present would be insufficient to cause an active response of the cells. On the other hand, a considerable voltage drop between the dorsal (apical) and the ventral (basal) surfaces of the epiblast has been demonstrated (MacKenzie, 1980; Stern & MacKenzie, 1983). This voltage amounts to more than 15 mV, and is many times greater than that required to cause electrophoretic redistribution of charged

molecules within the plane of the membrane of other cells (Jaffe, 1977; Poo & Robinson, 1977; Orida & Poo, 1978; Poo, 1979). The morphogenetic significance of transepithelial potentials has not been studied in any great depth in any system. Studies so far have concentrated mainly on current flow between cells within the tissues (via gap junctions) or *lateral* voltages along the extracellular spaces. I would like to suggest a way in which transepithelial voltages could lead to a break in the symmetry of the embryonic sheet.

This paper attempts to bridge some of the gaps which exist between different approaches to the problem of the control of early development. In applying different techniques, workers have developed different points of view to one another, and the resulting mass of information is rather disjointed. In the discussion that follows I shall suggest a few connecting strands which may provide experimenters with ideas to exploit the areas *between* those already fashionable.

Initial Conditions

The sodium pump probably causes an accumulation of sodium and water in the space between the epiblast and the forming endodermal layer (New, 1956; Howard, 1957; Stern, 1982*a*; Stern & MacKenzie, 1983) (see Figs 1(a)–(b) and 2(a)). The presence of this cell layer and of a basal lamina and extracellular materials under the epiblast (Sanders, 1979; Wakely & England, 1979; Vanroelen, Vakaet & Andries, 1980*a, b, c*; Alberts *et al.*, 1983) may be important in maintaining this concentration difference between the two sides of the epiblast.

Positive and Negative Controls

As the asymmetry of sodium concentration, voltage and hydrostatic pressure becomes more marked across the epiblast as a result of continued transport, it follows that cells would find it increasingly difficult to pump against an ever steeper electrochemical and hydrostatic gradient. It has been shown in other transporting epithelia that when the serosal (basal) hydrostatic pressure (Hsieh, Hsu & Lu, 1972; Voute, Mollgard & Ussing, 1975; Pequeux, 1976; Ziegler, 1977; Wilkins, 1979) or the serosal concentration of sodium or the transepithelial potential (Walser, 1972; Finn & Hutton, 1974; Cuthbert & Shum, 1976; Ziegler, 1977) increase above threshold values, the sodium pumps cease to pump, both the trans-epithelial short-circuit current and the trans-epithelial resistance decrease and inter-cellular junctions open (Fig. 2(b)). In the case of the chick epiblast, a

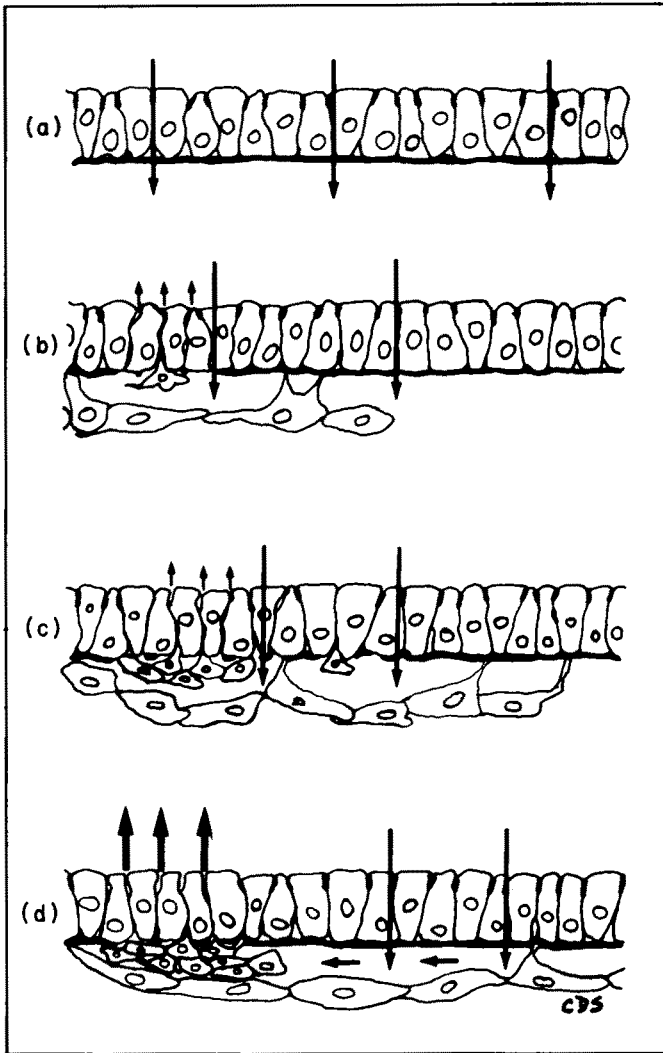


FIG. 1. Schematic diagrams showing the major postulated events during gastrulation in the chick embryo. Each diagram represents a section through the embryo. The posterior end of each section lies towards the left, dorsal (apical) towards the top and ventral (basal) towards the bottom. The epiblast is therefore at the top of each diagram, and within this tissue the intercellular tight junctions and the basal lamina are shown. The larger cells at the bottom of each diagram are the cells of the endodermal layer (hypoblast), whilst the smaller ones between the other two layers are the mesodermal cells. The arrows represent the direction of flow of sodium and water.

disruption of its continuity would lead to leakage of sodium and water out of the embryo and a return to sub-threshold conditions (Fig. 2(b)). The tissue would then resume pumping in the original direction (Fig. 2(c)). Thus the epiblast pumps may be subject to a negative feedback control which would set an upper threshold to their action. Additionally, it is possible that whilst this threshold is not attained, the system is also under positive feedback by electrophoretic localization of the pumps (see Jaffe, 1977), which like most membrane proteins are negatively charged, towards the inner (basal) side of the sheet, thus ensuring that pumping continues towards the interior of the embryonic space. This combination of controls would set the system oscillating between the two states.

The precise mechanisms connecting high serosal hydrostatic pressure, transepithelial voltage and transepithelial sodium concentration gradients with epithelial continuity in transporting tissues are not known. Transient breaks in the continuity of the epiblast have been seen in time-lapse films (Stern & Goodwin, 1977 & unpublished observations; Robertson, 1979) as vigorous focal contractions of the tissue, and in the epicentre of each contraction cells can sometimes be seen to leave the sheet. As many as 20 epiblast cells have been seen to disappear into the interior of the embryo in one of these contractions. This has also received support from electron microscopical observations (Bancroft & Bellairs, 1974; Fabian & Eyal-Giladi, 1981; Weinberger & Brick, 1982) of "rosettes" or pits in the epiblast where the tissue has lost its continuity. Other studies (Azar & Eyal-Giladi, 1979; Stern & Ireland, 1981) have shown the presence of small groups of cells between the two layers near the posterior margin of the blastoderm *prior* to the formation of a visible primitive streak. These cells resemble the later mesoderm in their morphology in tissue culture (Stern & Ireland, 1981 and unpublished observations).

The observation that loss of epithelial continuity is (a) centred around a focal point and (b) vigorous and transient suggests that the cytoskeleton of the epithelial cells is involved in generating the contractions (see Wakely & Badley, 1982, for a description of the arrangement of the cytoskeleton in chick epiblast cells). Based on theoretical grounds and a large amount of experimental data from the literature, Odell *et al.*, (1981) have suggested that instabilities in epithelial sheets brought about by geometrical constraints would lead to just this sort of contraction of the cytoskeleton. The forces involved are postulated to be (a) the intracellular calcium concentration, (b) a gelation-solution reaction of the cytoskeleton in response to changes in intracellular calcium (see Taylor, 1981; Alberts *et al.*, 1983) and (c) mechanical propagation. The mechanical forces which result from a contraction stretch the cytoskeletal elements of neighbouring cells and this propa-

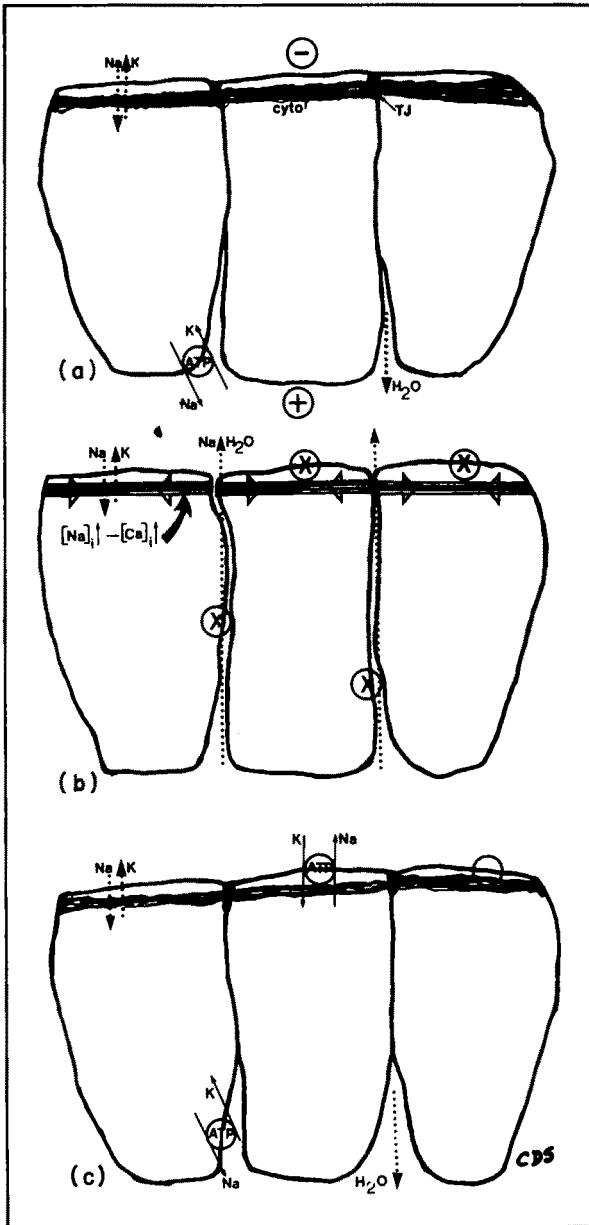


FIG. 2. Schematic diagrams showing some of the cellular details of ionic fluxes postulated to take place during gastrulation in the early chick embryo. Each diagram represents a section through three epiblast cells. Dorsal (apical) towards the top, ventral (basal) towards the bottom of each diagram. (a) Initial conditions. Sodium enters epiblast cells passively at the apical side

gates the reaction. If this series of processes has some sort of a "refractory period", this mechanism would preserve the location of the epicentre of the contractions.

Sodium and calcium transport are known to be interdependent in transporting epithelia (see Taylor, 1981). Changes in the level of cytosolic calcium lead to changes in the rate of transepithelial sodium transport. In turn, the entry of calcium is regulated by a process of sodium-calcium exchange (Taylor, 1981). A rise in intracellular sodium is known to induce calcium release from intracellular pools, causing a rise in intracellular free calcium (Lowe *et al.*, 1976). At the threshold point when the epiblast has ceased to pump (see above and Fig. 2(b)) the intracellular sodium concentration will rise since passive sodium influx into the cells is not affected (driven by a gradient of ~ 20 mM sodium inside cells versus ~ 100 mM outside; see Ziegler, 1977). The resulting rise in intracellular sodium concentration might trigger intracellular calcium release from bound pools (Lowe *et al.*, 1976) which could be the signal which triggers the initiation of cytoskeletal contraction (Fig. 2(b)). These connections between sodium, calcium and hydrostatic pressure may be the basis of the contractions in response to changes in transepithelial electrochemical gradients. After a contraction has been initiated in a group of cells, mechanical factors such as those suggested by Odell *et al.* (1981) might be involved in its propagation to neighbouring groups of cells.

These mechanisms would further ensure that the loss of epithelial continuity takes place only locally and in a transient manner.

Epithelial Continuity Versus Epithelial Polarity

Breakage of intercellular junctions can lead to redistribution (by diffusion) of membrane-contained molecules, as has been shown for dissociated epithelial cells (Pisam & Ripoché, 1976). It is therefore possible that the

of the tissue. Exit from the cells is via the Na-K-ATPase (sodium pump) at the baso-lateral side of the cells. Water follows passively in the same net direction as sodium, whilst potassium is taken up actively by the ATPase and leaves passively at the apical side of the tissue. cyto: cytoskeleton. TJ: intercellular tight junctions. (b) When the system reaches threshold (see text for details), the ATPase may cease to pump (x), and the continued intake of sodium leads to a rise in intracellular sodium levels which triggers calcium release. This increase in calcium triggers cytoskeletal contraction, whilst cells open their intercellular junctions allowing sodium and water to leak back to the apical side of the tissue, restoring equilibrium. The opening of the junctions allows movement of some of the sodium pumps towards the apical side of the tissue, perhaps aided by the basal-to-apical current between the cells. (c) Equilibrium has now been restored, pumping is once again occurring, but some of the pumps are now located at the apical side of the cells, transporting sodium in the opposite direction to the initial transepithelial flow.

transient breaks in the continuity of the epiblast sheet lead to local "scrambling" of some membrane-associated molecules (e.g. basal lamina components associated with the membrane, the ion channels and pumps themselves, cytoskeletal insertion points, etc.) (Fig. 2(b)). Interestingly, such a change in the position of various morphological polarity markers has indeed been shown at the primitive streak by various investigators. Vanroelen *et al* (1980*a, b, c*) have shown the presence of extracellular materials, which are normally situated basally in the epiblast, over the apical region of the groove of the primitive streak. Similarly, Mitrani & Farberov (1982 and unpublished) have shown that fibronectin and laminin both appear basally in lateral epiblast but not at all at the streak, and Stern (1982*a*) and Stern & MacKenzie (1983) have shown that ouabain binding sites appear basally in lateral regions of the epiblast but apically at the primitive streak. Furthermore, I have also recently shown that applying artificial standing voltages of the same magnitude but reverse polarity as those measured across the epiblast leads to rapid and stable reversal of the morphological and physiological polarity of the epiblast cells (Stern, 1982*b*; Stern & MacKenzie, 1983).

As mentioned above, sodium is pumped out of the primitive streak region (Jaffe & Stern, 1979; Stern, 1982*a*) and standing voltages of reverse polarity across the epiblast reverse the morphological and physiological polarity of the tissue (Stern, 1982*b*; Stern & MacKenzie, 1983). These two observations imply that the reorganization of the polarity of cells at the primitive streak may not be due simply to diffusion within the membrane. The local change in the direction of current flow (between cells at regions of epithelial disruption) may "actively" move membrane molecules as suggested by Jaffe (1977). This would account for the reversal, rather than random, distribution of membrane-associated polarity markers (basal lamina, sodium pumps, cytoskeleton, etc) at the primitive streak.

Epithelial Continuity and Epithelial-Mesenchymal Interactions

The endodermal layer (hypoblast) coalesces into a sheet in the presumptive caudal to cephalic direction (Eyal-Giladi & Kochav, 1976) and delimits a small cavity under the epiblast. If the presence of this cavity generates (or even just slightly increases) the sodium, voltage and hydrostatic pressure asymmetry across the epiblast, the contractions would tend to begin near the future posterior end of the embryo, where the cavity first arose (Fig. 1(b)). Thereafter, the location of the epicentre of the contractions would preserve itself (see below). It follows that the accumulation of the "de-epithelialized" (Vakaet, Vanroelen & Andries, 1980) cells arising from

the contractions would also be expected to be greatest near the future posterior end of the embryo (Fig. 1(b), (c)).

"De-epithelialized" cells such as these are found in later stages of chick embryo development in association with regions where the basal lamina of the epiblast is absent and where the structure of the adjacent epithelium is less cohesive, such as at the primitive streak (Vakaet *et al.*, 1980). It is possible that the absence of a basal lamina and the lowered cohesion are due to an ability of de-epithelialized cells to locally hydrolyse the overlying basal lamina. This is supported by recent work of Smith & Bernfield (1982) who found that mesenchyme cells can degrade the basal lamina underlying mouse submandibular salivary gland epithelium. In the early chick embryo, measurements of hyaluronidase activity in mesoderm and primitive streak cells revealed greatly increased levels of activity of this enzyme as compared to other tissues (Stern, 1983). Sanders (1980) has found that mesoderm is able to spread rapidly on the substrate-attached extracellular matrix of fibronectin which is secreted by other early chick embryonic tissues *in vitro*. Martinez-Palomo's (1970) study of the cell coat by electron microscopy shows (see Fig. 8 in Martinez-Palomo, 1970) a much reduced coat where mesoderm cells contact the basal surface of the epiblast. Similar results were obtained by Morriss & Solursh (1978), Vanroelen *et al.* (1980*a,b,c*), Manasek (1975) and Bernfield (1981), all of whom showed a much reduced glycosaminoglycan matrix surrounding mesoderm cells compared to that around the ectoderm in rat and chick embryos at primitive streak stages. Autoradiographical localization of the NaK-ATPase in the early chick embryo (Stern 1982*a*) showed that the region at which the ouabain binding reverses from the basal to the apical side of the epiblast coincides precisely with the distal edge of the mesoderm advancing out of the primitive streak.

One important function of this hydrolytic property of mesoderm cells may be the removal of the adhesive extracellular matrix which might otherwise *prevent* their migration laterally out of the primitive streak.

Autocatalysis and Overall Control

The generation of local contractions ("leaks") in the epiblast can therefore be viewed as an autocatalytic process. There would be two forces to achieve this: (1) as mentioned above, the contractile mechanism itself may have an inbuilt control to ensure that the location of the epicentre is preserved; (2) as the number of mesodermal cells increases in a particular region of the embryonic disc, so does the ability of this group of cells to degrade the basal lamina of the epiblast. The overlying tissue therefore becomes more

mechanically unstable, and the likelihood of more leaks occurring in the same region increases. The permeability of the epiblast to charged ion species and water may be lowered: the basal lamina tends to be permselective, as hyaluronic acid has a high affinity for ions such as sodium and it can change its conformation depending on the ionic environment (Phillips, 1970; Matthews & Decker, 1977; Chakrabarti, 1977). Furthermore, the activity of hyaluronidase itself is dependent on ionic strength and pH (Gorham, Olavesen & Dodgson, 1975; Doak & Zahler, 1979; Gacesa *et al.*, 1979).

Despite conflicting reports (see Comper, 1977), it is possible that hyaluronic acid matrices may partly act as mechano-electrical (i.e. piezoelectric) transducers (Barrett, 1975). This phenomenon may provide an interesting link between the transporting and mechanical properties of the tissue.

The Break in Symmetry and Formation of the Embryonic Axis

Autoradiographical evidence (Stern, 1982a; Stern & MacKenzie, 1983) showed that by the time the primitive streak has become established sodium pumps are located apically in the immediate vicinity of this structure, which would ensure that sodium is *driven* out of the embryonic cavity via the primitive streak. A more permanent pathway for the leakage of sodium and water would thus be established in this region, which would by then contain a considerable number of the mesodermal cells (Fig. 1(d)). This more permanent pathway would stabilize the ionic and hydrostatic situation in the embryo and become the primitive streak itself. It would thus also serve the function of preventing the formation of supernumerary axes elsewhere in the embryo. The cells engulfed in it during its formation would be the first mesodermal cells of the embryo.

The question then arises as to what forces determine the shape of the primitive streak. The above mechanisms account for the formation of a discrete area where mesodermal cells accumulate, but do not predict its shape. The nature of these forces is unknown, but it seems likely that two distinct but perhaps connected mechanisms may be taking part: (1) tension from the expanding edge of the blastoderm (Bellairs, Bromham & Wylie, 1967; Downie, 1976), and (2) pressure from converging morphogenetic movements of cells in the epiblast (Vakaet, 1970; Nicolet, 1971; Stern, 1978).

Application to Early Developmental Events

REGULATION

The above scheme of events accounts for the observation that a cut transecting the early embryo allows the formation of supernumerary axes

(Morita, 1936, 1937; Spratt & Haas, 1960). Since the space between the germ layers closes at the edges of the wound due to the great healing capacity of the germ layers (see England & Cowper, 1977), each transected piece simply forms its own cavity, within which the whole process of axis formation can take place.

The primitive streak always arises in the immediate vicinity of the margin between the central *area pellucida* and the peripheral *area opaca* (Spratt & Haas, 1960). This may imply that geometrical or other constraints (such as differences in cytoskeletal or epithelial structure between the two regions; see Wakely & Badley, 1982) increase the likelihood of the process of axis formation being initiated in this region.

PRIMARY INDUCTION

The postulated role of the mesoderm cells in disrupting the structure of the overlying basal lamina and the resulting alterations in permeability and mechanical properties of the epiblast would offer a basis for the primary inducing ability of the mesodermal cells. Such a mechanism may explain why mesoderm cells can induce the formation of supernumerary embryonic or neural axes when grafted in contact with epiblast into a host embryo. This mechanism would also account for the finding that a large variety of chemical and other stimuli are all capable of neural induction (see Gallera, 1971; Hara, 1978 for references). The present ideas therefore lead to the prediction that most of these agents will be characterized by their effects on the mechanical integrity and/or the permeability of the epiblast.

OTHER VERTEBRATES

Similar mechanisms for the formation and maintenance of the embryonic axis could operate in other vertebrate embryos. Bennett & Trinkaus (1970) found very similar current flow to that of the chick in the *Fundulus* embryo. In Amphibian embryos, the sodium pump has been shown to be important in neural development and earlier stages (Barth & Barth, 1969; Slack & Warner, 1973; Messenger & Warner, 1979), and mammalian (rabbit) embryos have recently been shown to have an asymmetric distribution of ouabain binding sites consistent with unidirectional sodium pumping, and a strophanthidin-sensitive trans-trophectodermal potential (Benos, 1981). The sodium transporting functions of rabbit embryos become particularly active just prior to gastrulation. Mouse embryos transport water into the interior of the blastocyst, which leads to visible regular expansion and contraction of its volume (Cole, 1967). These expansion-contraction oscilla-

tions may represent the mammalian equivalent of the chick contractions described above.

Epithelial/Mesenchymal Interconversion and Developmental Steps

The considerations in this paper point to a perhaps neglected aspect of the behaviour of tissues in developing organ systems. Organogenesis is often preceded by the conversion of an epithelium into a mesenchyme and from this back into an epithelium, this process repeating itself a variable number of times before "final" differentiation.

For example, some cells in the ectoderm lose continuity with the epithelium to form mesoderm, which then condenses into somites, which are epithelial spheres enclosing a lumen. Part of the somite then de-epithelializes to form the sclerotome, the mesenchymal cells of which migrate away from the somite, become perinotochordal, and differentiate. The myotome cells which are derived from the remaining part of the somite (the dermamyotome) later become mesenchymal themselves, migrate away from the somite and differentiate into muscle. Other mesodermal derivatives go through a similar series of steps; the intermediate mesoderm (that between the somites and the lateral plate) becomes epithelial to form the nephrotome (or nephrogenic cord), which will give rise to the kidney and its tubules, which are probably the transporting epithelium *par excellence*. The lateral plate mesoderm also becomes epithelial to form two sheets, the somatopleure and the splanchnopleure, which delimit the coelomic cavity. The mesoderm also gives rise to the blood vessels, which are epithelial. Neural crest cells, which can be considered mesenchymal, arise from the epiblast by de-epithelialization. Even primordial germ cells, which cannot be considered to be part of the endoderm during development, eventually lodge themselves within the germinal epithelia of the gonads, where they give rise to sperm or ova, which when released have many of the characteristic features of mesenchyme (low cell-to-matrix ratio, highly motile, etc.). Tissues which do not go through this interconversion exist, however; for example, skin (an ectodermal derivative) does not pass through a mesenchymal phase in its lineage.

Could the epithelial-mesenchymal interconversion represent a mechanism which "informs" the cells about the state of the environment *without* what is normally referred to as "cell communication"? Could it also be used as a coarse timing mechanism by which cells "count" conversions as a function of environmental conditions? Could it represent a simple way to regulate the size of developmental fields?

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