Morphogenetic behaviour of the rat embryonic ectoderm as a renal homograft

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SUMMARY

Halves of transversely or longitudinally cut primary ectoderm of the pre-primitive streak and the early primitive streak rat embryonic shield developed after 15-30 days in renal homografts into benign teratomas composed of various adult tissues, often in perfect organ-specific associations. No clear difference exists in histological composition of grafted halves of the same embryonic ectoderm.

The primary ectoderm of the pre-primitive streak rat embryonic shield grafted under the kidney capsule for 2 days displayed an atypical morphogenetic behaviour, characterized by diffuse breaking up of the original epithelial layer into mesenchyme. Some of these cells associated into cystic or tubular epithelial structures.

The definitive ectoderm of the head-fold-stage rat embryo grown as renal homograft for 1-3 days gave rise to groups of mesenchymal cells. These migrated from the basal side of the ectoderm in a manner which mimicked either the formation of the embryonic mesoderm or the initial migration of neural crest cells. This latter morphogenetic activity was retained in the entire neural epithelium of the early somite embryo but was only seen in the caudal open portion of the neural groove at the 10- to 12-somite stage.

The efficient histogenesis in grafts of dissected primary ectoderm and the atypical morphogenetic behaviour of grafted primary and definitive rat embryonic ectoderm were discussed in the light of current concepts on mosaic and regulative development, interactive events during embryogenesis and positioning and patterning of cells by controlled morphogenetic cell displacement.

INTRODUCTION

The space between the fibrous capsule and the parenchyma of the adult kidney offers a suitable environment for growth and differentiation of whole rat egg cylinders or separated germ layers. After a period of 15-30 days following transfer of such embryonic pieces, teratomas develop whose elaborate histological composition presumably reflects the developmental capacities of embryonic cells at the moment of transplantation (Škreb & Švajger, 1975; Škreb, Švajger & Levak-Švajger, 1971, 1976). Thus the developmental capacity...

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of the isolated rat embryonic ectoderm is greater if taken from the pre-primitive embryo streak than if removed from the head-fold stage of development (Levak-Svajger & Švajger, 1971, 1974). Towards the end of this developmental period the embryonic endoderm similarly displays regionally restricted capacities to differentiate into segments of the definitive gut when transplanted together with the adjacent mesoderm (Švajger & Levak-Švajger, 1974).

Similar results were obtained from testicular grafts of isolated germ layers of the mouse (Diwan & Stevens, 1976), thus indicating that the mechanism of definitive germ layer formation might be the same in both species. Moreover, this principle of gastrulation (origin of all three definitive embryonic germ layers from the primary ectoderm) seems to be even more general, for it has been observed also in the avian embryo, following the use of more direct methods (Nicolet, 1971).

The presumed morphogenetic movements of gastrulation in the rodent embryonic shield could be similar to the events in the chick blastoderm: a restricted area of extraordinarily rapid cell proliferation within the primary ectoderm (proliferative zone or centre) generates cells which, by expansion forces cause a considerable mass of cells to migrate through the primitive streak (Snow, 1976a, b, 1977). The primitive streak can be regarded as both the passageway for cells during gastrulation and the first, incomplete axis of bilateral symmetry of the embryo.

The temporally and spatially coordinated migration of cells through the primitive streak and the primitive node results in an ordered displacement of coherent cell sheets within the embryo. The final result of these morphogenetic cell movements is an orderly apposition of definitive germ layers to one another which makes possible inductive tissue interactions bringing about a spatial pattern of cellular differentiation and morphogenesis within the embryonic body.

When the early, pre-primitive streak embryonic ectoderm is transferred to an ectopic site such as the space under the kidney capsule, one can hardly expect that a regular primitive streak will form in this drastically altered physical environment. However, ectodermal, mesodermal and endodermal tissues regularly differentiate in these grafts and some tissues exhibit marked organ-specific cellular differentiation, of reasonably normal topography and spatial distribution (Škreba & Švajger, 1975). On the other hand, when at the head-fold stage the primitive streak and the primitive node regions are removed and only the anterior region grafted, mesodermal tissues (cartilage, bone, muscle) are regularly found in teratomas (Švajger & Levak-Švajger, 1976; Levak-Švajger & Švajger, 1979). Obviously, an adaptation of morphogenetic mechanisms to the ectopic environment should be presumed to exist in the transplanted ectoderm.

In order to shed more light on this problem the present investigation was undertaken to answer the following questions:

(a) Does the partial or complete removal of the primitive streak region in the early rat embryonic ectoderm essentially influence the degree, the diversity
and the organ-specificity of its histological differentiation as a renal homograft (series I, see Materials and Methods)?

(b) How do the future endodermal and mesodermal cells leave the primary ectoderm transplanted under the kidney capsule (series II)?

(c) How do mesenchymal cells originate and where do they come from in the renal grafts of the head-fold-stage rat embryonic ectoderm (series III)?

(d) At which developmental stage does the grafted embryonic ectoderm lose the ability to give rise to mesenchymal cells (series IV and V)?

**MATERIALS AND METHODS**

**Embryos**

Albino rats of the inbred Fischer strain were used in the experiments. Gestation was considered to have begun early in the morning when sperm was found in the vaginal smear. Twenty-four hours later the eggs were considered to be 1 day old.

Embryos belonging to the following post-implantation stages (after Witschi, see New, 1966) were used:

- **Stage 11** (gestation day 8): the pre-primitive streak (pre-gastrula), two-layered embryonic shield.
- **Stage 12** (gestation day 8½): the early primitive streak (early gastrula), start of mesoderm formation.
- **Stage 13** (gestation day 9): head-fold (late gastrula), head process, neural plate.
- **Stage 14** (gestation day 9½): neural groove, somites 1-3, start of foregut formation.
- **Stage 15** (gestation day 10): partly closed neural tube, somites 10-12, first aortic arch.

Pregnant females were anaesthetized with ether and the entire conceptuses (embryo + extraembryonic parts) were isolated from uteri with watchmaker's forceps in sterile Tyrode's saline. All extraembryonic structures were carefully removed and the isolated embryos were subjected to further manipulation.

**Isolation of the embryonic ectoderm**

The embryonic ectoderm was isolated from the pre-somite embryonic shields (stages 11, 12 and 13) and the early somite embryos (stage 14) by using the standard procedure for the separation of germ layers (treatment with proteolytic enzymes at 4 °C + microdissection) described in detail by Švajger & Levak-Švajger (1975). The stage-15 embryos (10-12 somites) were cut transversely into three segments before the treatment with enzymes and mechanical manipulations. According to the design of each experimental series, the isolated ectoderm was further dissected into pieces to be used as grafts.
Fig. 1. Schema of dissection of the pre-primitive streak and the early primitive streak rat primary ectoderm by transverse or longitudinal cuts (Experiment series I). ···, Contours of removed extra-embryonic parts; ——, cut for removal of extra-embryonic parts; ———, cut through the embryonic ectoderm.

Transplantation and fixation of grafts

Isolated pieces of embryonic ectoderm were transferred by means of a braking pipette under the capsule of the right kidney of an adult (3 months) male rat of the same strain. The recipient animals were killed by ether 1–30 days after transplantation. Grafts were excised with a razor blade together with a block of adherent host renal tissue. Very small grafts (fixed 1–2 days following transfer) were dissected out after a 30-min prefixation of the whole graft-bearing host kidney.
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Series II Stage 11

Series III Stage 13

Profile and en face view of the isolated part of the ectoderm

Series IV Stage 14

Cranial neuropore

Closed neural tube

Series V Stage 15

Caudal neuropore

Fig. 2. Schema which shows the origin of ectodermal areas which were grafted for 1–2 days to observe the morphogenetic behaviour of ectodermal cells (Experiment series II–V).

Histological procedures

(a) Paraffin sections. Grafts were fixed in Zenker’s fluid, embedded in paraffin wax, serially sectioned at 7 μm and stained with haemalum and eosin.

(b) Semi-thin sections. Grafts were fixed for 1 h in a mixture of 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer at 4 °C. They were washed in the same buffer and then post-fixed for 1 h in 1% osmic acid in the same buffer. Serial ethanolic dehydration was followed by embedding in Durcapan (Fluka). The 1 μm serial sections were stained with toluidine blue.
**Design of experimental series**

The work was divided into five series of experiments characterized by the developmental stage of embryos used, the region of the ectoderm used as graft and the period of cultivation in the host kidney. The purpose of each series was explained in the Introduction.

**Series I. Halves of pre-gastrula and early gastrula embryonic ectoderm.** Embryonic ectoderm belonging to developmental stages 11 (pre-primitive streak) and 12 (early primitive streak) was divided approximately into two halves by either a transverse or a longitudinal cut (Fig. 1). Longitudinal cuts were made haphazardly, with no respect to the expected (stage 11) or actual (stage 12) position of the primitive streak. Each half was transplanted separately under the kidney capsule. In 23 of the transversely cut stage-11 embryonic shields the primary endoderm was not removed from the ectoderm. The halves of the stage-12 ectoderm were transplanted together with the mesodermal wings emerging from the primitive streak. The grafts were fixed after 15–30 days and examined histologically for the presence and distribution of mature tissues (Total number: 88 embryos = 176 grafts).

**Series II. Whole pre-gastrula embryonic ectoderm.** The primary ectoderm of the pre-primitive-streak (stage-11) embryo was transplanted and fixed after 2 days (23 grafts, Fig. 2).

**Series III. Incomplete late gastrula embryonic ectoderm.** The posterior part and the tip of the stage-13 egg cylinder (areas containing the primitive streak and the primitive node respectively) were cut off prior to the germ layer separation procedure, and the rest of the embryonic ectoderm was transplanted for 1–3 days (152 grafts, Fig. 2).

**Series IV. Incomplete early-somite-stage ectoderm.** As in the previous series, the primitive streak and the primitive node regions were removed from the stage-14 embryo. The isolated and grafted ectoderm roughly corresponded to the neural fold. It very probably contained presumptive areas of both the neural epithelium and a part of the surface ectoderm (future epidermis) which are not yet sharply demarcated at this stage (Waterman, 1976; ectoderm classes II–V of Verwoerd & van Oostrom, 1979; 13 grafts, Fig. 2).

**Series V. Neuroectoderm of the stage-15 embryo.** Prior to treatment with enzymes the whole embryo was transversely cut into three segments which contained: (a) the cranial neuropore (from which the rostral part with the optic

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Fig. 3. Origin of thymus (T) from the foregut (F) epithelium. Experiment series I. H.E. x 100.

Fig. 4. Respiratory tract differentiation and morphogenesis in the teratoma. T, trachea; B, bronchial bifurcation; L, lung lobe. Experiment series I. H.E. x 50.

Fig. 5. A complex structure reminiscent of the foetal mandible, developed in the teratoma. T, tooth germ; B, membrane bone; C, hyaline cartilage. Experiment series I. H.E. x 70.
vesicles was removed), (b) the closed neural tube, and (c) the caudal neuropore (from which the tail bud with remnants of the primitive streak was removed). After treatment with enzymes the closed neural tube was cleanly separated from the overlying surface ectoderm. The thick neuroepithelium of the cranial and caudal neuropore was easily isolated from its continuous surface ectoderm along the distinct demarcation line (Waterman, 1976). Each of the three segments of the neuroectoderm was separately transplanted and fixed after 2 days (32 grafts, Fig. 2).

RESULTS

Series I (Figs. 3–5). Halves of the pre-primitive streak and the early primitive streak embryonic ectoderm transplanted to the host kidney for 15-30 days developed into solid tumours of various sizes, comprising a multitude of well-differentiated tissues, often arranged in a clearly recognizable organ-specific association. The chaotic arrangement of adult tissues conformed to the definition of benign or mature embryo-derived teratoma (Damjanov & Solter, 1974; Solter, Damjanov & Koprowski, 1975).

Tissues found in grafts were derivatives of all three definitive germ layers:

Ectodermal tissues: skin (epidermis, hairs, sebaceous and mammary glands), neural tissues (brain, neural retina, choroid plexus, ganglia) and other ectodermal derivatives (lentoids, oral cavity with teeth, and salivary glands).

Endodermal tissues: foregut-derived epithelia (glands, thymus, thyroid, parathyroid, oesophagus, stomach, respiratory tube, lungs), mid- and hindgut-derived epithelia (small and large intestine, urogenital sinus, prostatic gland).

Mesodermal tissues: white and brown adipose tissues, cartilage, membrane and enchondral bone, smooth, skeletal and heart muscle).

Well-expressed organ-typical differentiation and combinations of tissues were regularly observed. Endoderm-derived epithelia displayed a wide range of segment-specific differentiations (stratified squamous epithelium of the oesophagus and forestomach, typical surface and glandular epithelium of the glandular stomach, small and large intestine, pseudostratified ciliated epithelium with a continuous transition into the epithelial lining of the lobe-shaped lung (Fig. 4). Glandular derivatives of the primitive gut always showed a typical regional origin: thymus and thyroid originated from the foregut epithelium (Fig. 3) while the prostatic gland appeared in direct continuity with the urogenital sinus, which was closely topographically related to the large intestine. The pattern of cellular differentiation in mesodermal tissues also displayed distinct organ-specificity. The pseudostratified ciliated epithelium of the respiratory tube was thus associated with pieces of non-ossifying hyaline cartilage, while the stomach and intestinal epithelium was surrounded by two layers of smooth muscle. Small ganglia were often found in close proximity of the muscular intestinal wall or even between the two muscular layers (intramural ganglia). Among especially peculiar tissue combinations, found only in single
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grafts were: a tooth germ surrounded by membrane bone and pieces of hyaline (Meckel's?) cartilage, mimicking the whole foetal mandible, and the optic cup enclosing a mass of lentoid cells. Interestingly, in the later case lentoid cells did not originate from the surface ectoderm, but from cylindrical epithelium which most probably belonged to the anterior edge of the optic cup. The whole tissue complex was thus strongly reminiscent of the Wolffian lens regeneration in amphibians.

Several difficulties, most of them of a technical nature (lower rate of successful grafts of halves when compared with grafts of whole embryonic shields, random topographical distribution of cuts through the embryonic ectoderm) make it impossible to perform any exact numerical analysis of differences in the histological composition of grafts, especially of pairs of grafts (halves of the same embryonic shield). Therefore only the following general statements can be made:

(a) Each graft, regardless of its origin, contained adult tissues derivatives of all three definitive germ layers.

(b) Neural tissue was present in all grafts, and therefore in both grafts of a pair.

(c) The tissue composition of particular grafts varied a great deal, but without any distinct prevalence of particular tissues in particular categories of grafts.

(d) Even tissues which probably originate from restricted areas of the primary ectoderm in situ (heart, respiratory tube, stomach, intestine, prostatic gland), could be found in grafts of both halves of the same embryonic ectoderm.

Series II (Figs. 6–9). The primary ectoderm of the pre-primitive streak rat embryonic shield displayed, as a renal homograft, remarkable deviations of morphogenetic behaviour in comparison with its normal development in situ. By 2 days after transplantation the original, compact epithelial organization of the primary ectoderm was hardly recognizable (Fig. 6). The grafts still consisted of undifferentiated cells, but only a small part of them retained the epithelial configuration. In general, three types of cell associations could be discerned (Figs. 7, 8, 9): (a) sheets of tightly packed epithelial cells (remnants of the original ectoderm?), (b) sheets of cells which still retained the two-dimensional pattern of the epithelium, but the contact between neighbouring cells was loosened (these epithelial cells sometimes formed cystic or tubular structures with irregular outlines, Fig. 8), and (c) mesenchyme-like masses of loosely dispersed, irregularly outlined cells (Fig. 9). These cells originated from various portions of the grafted primary ectoderm, with apparently no respect to the topographical position of the primitive streak in situ. It is impossible to associate any of the observed cell assemblies with a final tissue and/or organ derivative.

Series III (Figs. 10–19). The head-fold-stage rat embryonic ectoderm continued to develop, as renal a homograft, into both the high pseudostratified columnar epithelium of the neural tube (neuroepithelium) and the simple, lower epithelium of the surface ectoderm or the future epidermis (classes IV–VI and I–III respectively of Verwoerd & van Oostrom, 1979). Immediately after grafting the ectoderm was either extended as a flat sheet or folded with its basal
Fig. 9. Detail of the primary ectoderm 2 days after transplantation. Note the diffuse breaking up of the ectoderm into mesenchyme. Mitotic figures (arrow) and dead cells (arrowhead) are also seen. K, host kidney tubules; Ca, renal capsule. Experiment series II. Semi-thin section. × 600.

side outwards. Two or three days later it kept this original shape or gave rise, at least partly, to cystic, tubular or rosette-like structures (Figs. 10–12, 17, 18). Each of these epithelial forms seems to produce groups of cells, roughly similar to mesenchyme. These cells are always found on the basal side of the epithelial sheet, regardless of whether it was apposed to the parenchyma or the capsule of the host kidney, or to the periphery of neighbouring epithelial cysts or tubules.

Two ways could be distinguished by which this ‘mesenchyme neoformation’ seems to take place: (a) breaking up or dissociation of a portion of the epithelium into an amorphous group of loosely dispersed cells (as in the previous experimental series), and (b) protrusion of groups of more closely packed cells (often in the form of tongue-like projections) beyond the basal boundary of the epithelium. The first way seems to occur more generally, starting from all the above listed epithelial forms of the grafted ectoderm (Figs. 11, 12, 17, 19). The

Fig. 6. General appearance of the primitive ectoderm (E) 2 days after transplantation. K, host kidney. Arrows point to the sharp boundary between graft and host tissues. Experiment series II. H.E. × 300.

Fig. 7. Detail of the primary ectoderm 2 days after transplantation. K, host kidney; Ca, renal capsule. Note mesenchyme and epithelial structures. Experiment series II. Semi-thin section. × 600.

Fig. 8. Detail of the primary ectoderm 2 days after transplantation. Note the irregularly outlined epithelial tubule (T). Experiment series II. Semi-thin section. × 550.
second way is strongly reminiscent of the initial migration of neural crest cells \textit{in situ} (Morriss & Thorogood, 1978). It regularly occurs within small areas on the basal surface of a thick ectoderm, which is most probably equivalent to the neural plate (Figs. 13–16, 18, 19). Interestingly enough, this way of cell migration could sometimes be observed in grafts just at the boundary between the thick
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Fig. 13. Protrusion of a tongue-like projection (arrow) from the basal side of the head-fold stage embryonic ectoderm, 2 days after transplantation. Experiment series III. H.E. × 200.

Fig. 14. A higher magnification micrograph of a portion of Fig. 13. Note the outgrowth of cells (arrow). E, ectoderm. H.E. × 520.

Fig. 15. Outgrowth of closely packed cells from the basal side of the head-fold stage embryonic ectoderm, 2 days after transplantation. Experiment series III. H.E. × 200.
and the thin ectoderm, thus providing an almost exact copy of neural crest development \textit{in situ} (Fig. 16).

Roughly estimated, the mitotic activity of grafted ectodermal cells did not differ essentially from its counterpart \textit{in situ}. Mitoses could be observed predominantly within the innermost (ependymal) layer of the thick ectoderm. Dead cells were also a common finding. In some grafts massive cell death was observed in both the grafted ectodermal layer and the newly formed mesenchyme cells.

\textit{Series IV.} Two days after transplantation the ectoderm of the early-somite-stage rat embryo still gave rise to new mesenchymal cells although to a considerably reduced extent and predominantly in a form reminiscent of neural crest formation \textit{in situ}.

\textit{Series V.} Two days after transplantation grafts of the closed neural tube and of the cranial, open neural groove formed relatively thick, disk-shaped and sharply outlined tumours which consisted exclusively of differentiating neural tissue with outgrowing axons and glial cells.

On the other hand, grafts of the caudal open neural groove were thin and the degree of neural tissue differentiation within them was less advanced. The outline of these grafts showed local irregularities with outgrowth of small groups of cells.

\section*{DISCUSSION}

\textit{General remarks on ectopic development of early mammalian tissues}

The whole experimental procedure applied in this or in similar experiments, involves a number of atypical influences upon the isolated embryonic tissue, the effects of which are poorly understood or completely unknown. These are: (a) temporary interruption of blood circulation, (b) influence of environmental constituents (saline, serum, enzymes), (c) surgical trauma, (d) loss of connexion with extraembryonic membranes, (e) altered physical (spatial) conditions after transplantation, (f) reduced supply of oxygen and nutrients before full vascularization of grafts, and (g) interactive influences from the host tissues. The basement lamina, which already exists between germ layers of the early embryonic shield (Adamson & Ayers, 1979; Pierce, 1966) is dissolved during the treatment with enzymes. Very probably, proteolytic enzymes can also considerably affect the composition and properties of the cell surface. The problem is best revealed by quoting Waymouth (1974): 'It is doubtful whether carefully controlled enzyme treatments are less traumatic than cutting, pressing, or
otherwise mechanically reducing tissues to manageable size for explantation. Any honest practitioner of cell and tissue culture will acknowledge that his art is one of survival of fittest cells in conditions that are never quite ideal'. Snow (1976a) pointed to the possibility that developmental capabilities of the isolated embryonic tissues may be modified by surgical trauma. In the present study mechanical distortions observed in some grafts fixed 2 days following transfer, have most probably arisen during the histological procedure. Morphogenetic features within the grafts did not show any particular relationship to these defects.

Prior to isolation the free and the basal surfaces of the ectoderm are in contact with the amniotic fluid and the basal lamina respectively. After transplantation under the kidney capsule the graft's new environment consists of loose connective tissue and, at the capsular side, of one or more layers of peculiar squamous cells (Bulger, 1973). We do not know whether this atypical microenvironment exerts any significant influence upon the graft. As pointed out in a previous...
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paper (Levak-Švajger & Švajger, 1974), the great diversity of differentiation and organ-specific tissue associations in teratomas is unlikely to have been non-specifically induced by the host tissue. In the same study the presence of tissue derivatives of particular germ layers varied regularly in relation to the original germ-layer composition of the graft. It is therefore most probable that the final composition of the grafts reflects their initial developmental capacities.

Differentiation of parts of the dissected primary ectoderm

(Experiment series I)

The unusually shaped rat and mouse egg cylinder with its inverted germ layers presents considerable difficulties in orientation and manipulation. A preliminary testing of developmental capacities of isolated parts of the rat embryonic ectoderm (Švajger & Levak-Švajger, 1976) showed that the borderlines between the frontal and lateral ectoderm, arbitrarily chosen in the mouse egg cylinder by Poelmann (1980) do not sharply delineate ectodermal regions with neural and surface ectodermal (epidermal) differentiative capacities. Although a regionalization with respect to mitotic activity has been demonstrated within the mouse epiblast (Snow, 1977), a detailed fate map of presumptive areas, as existing for the chick blastoderm (Rosenquist, 1966), can hardly be imagined in the embryonic shield of rodents. Moreover, the exact position of the primitive streak is difficult to foresee or even to record at its early stages.

With all this in mind the random transverse or longitudinal cutting of the early egg cylinder into two approximately equal parts seems to be a very simple and unpromising experimental design. After transverse cutting, the part of the cylinder adjacent to the extraembryonic membranes contained regions corresponding to the anterior portion of the neural plate and the posterior end of the primitive streak. The other part (with the tip of the cylinder) contained regions corresponding to the posterior portion of the neural plate and the anterior end of the primitive streak. The random longitudinal cutting resulted most probably in one half containing the primitive streak region and the other without it. One might think that in both experimental designs the two halves of the same embryonic ectoderm differed in the (partial) presence or absence of the primitive streak and the presumptive organ-forming areas. However, mature tissues, often in normal organ-specific associations, developed in all grafts, regardless of the variations in the initial developmental stage and the direction of the cut. These results suggest that within the primary ectoderm areas with different developmental potentialities are not yet sharply demarcated or, at least, that the revealing of regionally restricted prospective areas is highly complicated in the inverted egg cylinder. The other essential conclusion is that cells with endodermal and mesodermal destinations can leave the primary ectoderm in regions other than the usually positioned primitive streak.

An obvious question is, how are the specific epitheliomesenchymal interactions established in the absence of coordinated displacements of future
endodermal and mesodermal cells through the primitive streak? This might, however, not be surprising if one remembers that even at later developmental stages factors other than strong local specificity are involved in these interactions (Lawson, 1974), and that non-cellular substrata may substitute for the local mesenchyme in supporting differentiation of digestive tract epithelia in chickens (Sumiya, 1976).

Atypical morphogenetic behaviour of the primary ectoderm
(Experiment series II)

During normal development in situ the formation of the primary mesenchyme from the primary ectoderm can be defined as the movement of individual cells in a migrating cell stream using the primitive streak as the passageway (Solursh & Revel, 1978). The atypical behaviour of the primary ectoderm as an explant, observed in this study, can best be defined as the 'breaking up of epithelial layers to produce mesenchyme' (Balinsky, 1975). The general appearance of this process is very similar to the partial dissociation of the epithelial somite into sclerotome (secondary mesenchyme, Hay, 1968). One might speculate that the ectoderm possesses an intrinsic tendency to produce new cell layers. After transfer to an ectopic site the onset of this activity takes place in remarkably disturbed environmental conditions. The basal lamina, whose collagen probably 'acts as a railroad track to guide the migration of the primitive streak mesenchyme' (Hay, 1973), is dissolved by enzymes, and the epithelial ectodermal sheet is tightly trapped within the subcapsular space of the host kidney. Very probably both these circumstances may account for the deviation from the normal mechanism of primary mesenchyme production. In this context, it is interesting to note that in early mouse embryos cultivated in vitro, in conditions which allowed the expansion of embryonic and extraembryonic cavities, mesoderm formation occurred through a primitive-streak-like structure (Wiley & Pedersen, 1977; Wiley, Spindle & Pedersen, 1978; Libbus & Hsu, 1980), whereas the abortive mesoderm formation in cystic embryoid bodies proceeds in a modified way. Unfortunately the available data do not permit a clear comparison with the mechanism observed in the present study (Stevens, 1960; Martin, 1977; Martin, Wiley & Damjanov, 1977).

Cystic and tubular structures in explants were usually lined by epithelial cells whose irregular shape and size, as well as the occasionally loosened intercellular contacts, gave the impression that they have arisen by aggregation of mesenchymal cells. However, the 2-day interval between transplantation and fixation of grafts was obviously too long for recording all the gradual changes within the explant. At this early stage the simple morphology of these epithelial structures does not permit any prediction about the future differentiation into neural, epidermal, intestinal or mesodermal epithelia. One may note that the development of atypical tubular epithelia of mesodermal origin was also
observed in T-mutant mouse embryos and interpreted as an aberrant kind of somite and notochord construction (Spiegelman, 1976).

Even more than in the previous series, it is difficult to reconcile this chaotically disturbed morphogenetic cell behaviour with the observed normal tissue differentiation and elaborate organ-typical association of older grafts. It is commonly accepted that the positioning and patterning of cells by controlled morphogenetic cell movements is a prerequisite for the construction of the basic structure and interrelationships of tissues. According to Curtis (1978) cell patterning can arise either by positioning of pre-differentiated cells or by differentiation of cells that have already taken up their final position. In the present case one might discuss the following two possibilities:

(a) The primary ectoderm is a heterogeneous population of small groups of pre-determined cells which already bear discrete cell surface properties necessary for future organ-specific cell–cell recognition. These cells and their immediate progeny could therefore recognize each other, associate (aggregate) and differentiate in a tissue- and organ-specific pattern regardless of the way in which they have left their original position within the primary ectoderm. In other words, the pre-determined cells may overcome the loss of their initial correct positioning and coordinated movement and find their 'required' final positions by mechanisms similar to those involved in tissue-specific sorting-out of cells from mixed aggregates *in vitro*. This principle of mosaic or polyclonal development, or 'development by means of compartments' (Garcia-Bellido, Lawrence & Morata, 1979) is consistent with some data on the early regionalization of the pre-gastrulation ectoderm in various classes of vertebrates. These include the findings of electrophoretically distinct subpopulations of cells within the undifferentiated amphibian ectoderm (Ave, Kawakami & Sameshima, 1968); of selective sorting-out of cells in aggregates prepared from unincubated chick blastoderms (Zalik & Sanders, 1974); and of regionalized mitotic activity in the mouse primitive ectoderm (Snow, 1977).

It is interesting that a mechanism analogous to that observed in this study, i.e. the formation of gut epithelium directly from the mesenchyme, operates in the tail region during the normal development (‘direkt gebildetes Entoderm’, Peter, 1941).

(b) The primary ectoderm is a homogeneous population of undetermined, pluripotent cells, which move away from their original positions and reach another place where 'first the cells are assigned positional information and then they interpret that information according to their genetic program’ (Wolpert, 1978). This regulative type of development, or differentiation in response to environmental factors, has been demonstrated in various systems in vertebrates. Among the most impressive examples are: tissue or organ-specific differentiation (or metaplasia) of epithelia of the avian and mammalian embryonic membranes in response to specific or non-specific environmental stimuli (Moscona, 1959; Moscona & Carneckas, 1962; Kato & Hayashi, 1963; Yasugi & Mizuno, 1974;
Payne & Payne, 1961), contribution of Schwann sheath cells in the regeneration of the salamander limb (Wallace, 1972), differentiation of cartilage from differentiated muscle cells (Nathanson, Hilfer & Searls, 1978; Nathanson & Hay, 1980), and the conversion of cell type or transdifferentiation occurring during the Wolffian lens regeneration in amphibians (Yamada, 1977). In addition the clonal contribution of single teratocarcinoma cells to normally differentiated tissues in chimaeric mice (Illmensee & Stevens, 1979), strongly suggests epigenetic influence on their development.

All these data, however, concern the plasticity of some cells in response to unusual environmental conditions, rather than the repertoire of potencies which are realized during undisturbed development in situ. In other words, the occasional expression of pluripotentiality or aberrant developmental tendencies by various differentiated cells does not necessarily rule out the existence of covert populations of pre-determined cells within the primary ectoderm. Any attempt to explain the atypical behaviour of these cells in renal explants in terms of either the pre-determination or positional information, is limited by major gaps in our knowledge about what is actually going on in embryonic cells as they pass along their developmental pathway. Even clear-cut experimental results might provide only suggestive rather than definitive data if we keep in mind that ‘we have practically no idea of what is really going on in cells of the blastoderm when they move, invaginate, induce or are induced, interact, become determined and begin to differentiate’ (Leikola, 1976), and that ‘we have no idea how positional signalling is accomplished or how cells record and remember their positional value’ (Wolpert, 1978).

Atypical morphogenetic behaviour of the definitive ectoderm

(Experiment series III–V)

The head-fold stage immediately follows primary induction and precedes neurulation, somitogenesis and primitive gut formation. Despite the remarkably advanced state of determination of the ectoderm, it still displays some of the atypical morphogenetic properties of the primary ectoderm: it breaks up into mesenchymal cells and forms cystic, tubular or rosette-like structures. New cells always originate from the basal side of the ectoderm, whether they are adjacent to the parenchyma or the capsule of the host kidney. This fact apparently implies an absence or lack of specificity of inductive influences by host tissues. The localized dissociation of the definitive ectoderm into a mesenchyme-like tissue might most probably be regarded as a residual capacity to form primary mesenchyme (embryonic mesoderm) even in the absence of the primitive streak. Determination of the ectoderm is therefore not yet fully stabilized and it would not be appropriate to designate the observed mesenchyme neoformation by terms such as cellular metaplasia, switch in differentiation, cell-type conversion or transdifferentiation (see Yamada, 1977).

The other form of mesenchyme production at this stage is reminiscent of
neural crest formation and is most probably equivalent to it. Both primary mesenchyme formation via the primitive streak and ectomesenchyme formation via the neural crest involved local conversion of an epithelial into a mesenchymal tissue organization, probably by the same cellular mechanism. Proper identification of the neural crest-like cells depends upon whether their origin is from the neuroectoderm and results in a more condensed organization of the mesenchymal derivative (Morriss & Thorogood, 1978). A similar, but not identical organization of the newly formed mesenchyme was observed in the arrested primitive streak region of the T-mutant mouse embryo (Spiegelman & Bennett, 1974). A preliminary histological examination of the same type of explant but fixed after 15 or more days, has revealed the presence of skeletal muscle in addition to hyaline cartilage and membrane bone. These data suggest that the newly formed mesenchyme in grafts of definitive ectoderm might be analogous to both the mesoderm of primitive streak origin and the mesectoderm of neural crest origin. However, before a detailed analysis little can be said about the real developmental capacities of the neural crest-like cells in the present experimental conditions, especially as during normal development in situ cells of neural crest origin differ in their dependence on post-migratory tissue interactions for differentiation into various typical derivatives (Hall & Tremaine, 1979).

The restriction of the mesenchyme-forming capacity of the ectoderm transplanted at a later developmental stage corresponds to the definitive stabilization of neuroectodermal and epidermal components in the course of neural tube closure. The caudal portion of the neural tube is the last one to lose this capacity. This is compatible with developmental features in situ, where the posterior end of the neural tube gives rise to the mesenchyme of the tail bud (Jolly & Férestet-Tadié, 1936).

It may be noted that an atypical, indistinct boundary between the closed neural tube neuroepithelium and the surrounding mesenchyme was also observed in T-mutant mouse embryos (Spiegelman, 1976).

**CONCLUDING REMARKS**

In the attempt to make more or less decisive conclusions about the observed phenomena we cannot avoid 'a problem that constantly faces the biologist, namely, that his concepts are limited both by the data on which he attempts to build them and by the design of the human mind' (Hay & Meier, 1978).

The main issue of this study is the finding that the primary ectoderm of the pre-gastrulation rat embryo can give rise to properly differentiated tissues and elaborate organ-specific tissue associations without the involvement of co-ordinated morphogenetic movements of cells through the primitive streak. In the ectopic environment it dissociates into mesenchyme-like cells which then apparently associate in a way reminiscent of the sorting-out and tissue-specific aggregation of embryonic cells from mixed suspensions. One may suppose that
the morphogenetic aspects of gastrulation are essential for the determination of polarity and for the complete organization of pattern in the embryonic body, but not for the establishment of histotypical and organotypical cell combinations.

The various aspects and implications of altered morphogenetic cell movements in ectodermal explants can hardly be unequivocally explained within the framework of commonly held views which direct our thinking about development. As an example, how can the elaborate tissue pattern of the intestinal wall be achieved from an amorphous cell mass, when a definite organization into distinct cell layers is apparently required for the subtle 'directive' and 'permissive' interactive events (Saxén, 1977) during embryogenesis in situ? But surprisingly, the same 'abnormal' mechanism is at work in the tail bud of the embryo which develops in utero! Also, when we think about the mosaic or regulative nature of a particular developmental event, we should bear in mind that even experimentally well argued concepts, such as that on the divergence of stable cell lineages within the mouse blastocyst (Adamson & Gardner, 1979), are valid only in terms of prospective significance, and tell us little of prospective capacity (metaplastic changes of the extraembryonic endodermal epithelia!). Features like lens regeneration by transdifferentiation might support this view.

Beliefs are being expressed that 'there may be an universal mechanism whereby the translation of genetic information into spatial patterns of differentiation is achieved' (Wolpert, 1969). In fact, hardly any developmental mechanism is fully understood yet, and therefore any search for universal mechanisms appears hopeless. In order to avoid giving new names to our ignorance we had better to join C. H. Waddington (1957) in his belief that 'It seems impossible to hope that we shall ever discover any single basic mechanism of pattern formation or morphogenesis, as we may still hope to find, for instance in the mechanism of protein synthesis and its control by genes, the fundamental mechanism for substantive differentiation. In discussing pattern formation and morphogenesis, therefore, one can hardly hope to do more than provide a number of illustrations of the general nature of the processes which are at work'.

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Morphogenesis in grafted rat ectoderm


A. ŠVAJGER AND OTHERS


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