REVIEW ARTICLE

Rat embryonic ectoderm as renal isograft

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INTRODUCTION

Experimental results obtained many years ago revealed that during gastrulation (with the primitive streak and the mesoderm formation as distinct features) the early rodent embryo undergoes essential changes in its response to extrinsic teratogens (Russell & Russell, 1954; Wilson, 1954; Škreb, 1961; Škreb & Bijelić, 1962; Škreb & Frank, 1963). It has also been shown that the ultrastructural, histochemical and biosynthetic features of the embryo are subject to substantial changes during this period (Solter, Damjanov & Škreb, 1970, 1973; Dziadek & Adamson, 1978; Bode & Dziadek, 1979; Wartiovaara, Leivo & Vaheri, 1979; Jackson et al. 1981; Franke et al. 1982a,b). This suggests a restriction of developmental capacities (i.e. the loss of the capacity of regulation) in groups of embryonic cells at this developmental stage.

According to the current concept, the initial cell population from which this restriction starts, resides within the embryonic ectoderm of the pregastrulation or preprimitive streak embryo (primitive or primary ectoderm). The first experimental evidence in favour of this view was contributed by Grobstein (1952). A more rigorous analysis has been made possible by the improvement of the technique of separation of germ layers (Levak-Švajger, Švajger & Škreb, 1969; Švajger & Levak-Švajger, 1975). The developmental potential of isolated germ layers of the rat embryo has been tested by grafting them under the kidney capsule of adult syngeneic animals and by histological analysis of the resulting teratomas (Škreb & Švajger, 1975; Škreb, Švajger & Levak-Švajger, 1976; Švajger, Levak-Švajger, Kostović-Knežević & Bradamante, 1981).

The space between the kidney capsule and the parenchyma of the adult kidney offers a suitable environment for growth and differentiation of both the whole rat egg-cylinder and the separate germ layers (Škreb & Švajger, 1975). However, even in this favourable ectopic site the transferred ectoderm undergoes considerable growth and differentiation.

Key words: rat embryo, renal isograft, ectopic grafts, kidney capsule.
changes in its morphogenetic behaviour when compared with its normal development in situ (Švajger et al. 1981). Here we review the essential results from experiments with renal isografts of rat embryonic ectoderm, compare them with the relevant results obtained in other experimental systems and give a critical evaluation of conclusions in the light of the atypical features which may occur in ectopic grafts.

TERMINOLOGY

Several synonyms have been used to designate the three germ layers: ectoderm (epiblast, ectoblast, ectophyll), mesoderm (mesoblast) and endoderm (hypoblast, endoblast, endophyll). The terms epiblast and hypoblast are commonly used for the primitive germ layers of the early chick embryo, while the terms ectoderm and endoderm are more common in mammalian embryology. The parallel or even combined usage of these sets of terms is confusing. It is therefore high time to decide to use a strictly unique terminology, at least for the mammalian embryo. It is important to distinguish between germ layers before gastrulation (the primitive or primary ectoderm and endoderm) and after the completion of gastrulation (definitive ectoderm, mesoderm and endoderm). The criteria are given in Table 1.

Without the adjectives 'primary' or 'primary' the terms ectoderm, mesoderm and endoderm (endo = ento) mean definitive germ layers. In connection with the mesoderm (which lacks a topographically analogous cell layer before gastrulation) the adjectives 'primary' and 'secondary' are sometimes used to designate the

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Table 1. Survey of rat embryonic germ layers
developmental stages of the *mesenchyme* as the first and most widespread tissue type of mesodermal origin. The mesodermal cells leave the primitive streak in the form of the primary mesenchyme, which then consolidates into provisory epithelial structures (somites, intermediary mesoderm, lateral plates). These in turn partly dissociate to form the secondary mesenchyme (sclerotome, dermatome, myotome, renal and lateral plate mesenchyme) (Hay, 1968).

The terms *proximal* and *distal* (ectoderm) refer to the position in relation to the amnion and other extraembryonic membranes, and the terms *anterior* (frontal), *posterior* and *lateral* refer to the position in relation to the embryonic axis (see Poelmann, 1980b; Snow, 1981; Beddington, 1983a; Tam & Meier, 1982; Tam, 1984).

The embryonic germ layers are continuous with those which make the extraembryonic membranes. The latter will not be considered in this text.

In order to simplify the explanation of some results reviewed here, we will sometimes use the adjectives ‘ectodermal’, ‘endodermal’ and ‘mesodermal’ with quotation marks to designate the tissues or cells which are supposed to derive from the corresponding definitive germ layers *in situ*. However, in view of the atypical morphogenetic events which occur in renal isografts of the isolated primitive ectoderm, it is actually meaningless to speak of the germ layer origin of tissue constituents of the resulting teratomas. In general, *the terminological designations of the germ layers will be used here in their classical, topographical meanings, which do not imply their actual potencies for tissue differentiation in situ and in various experimental conditions.*

**MANIPULATION OF EMBRYOS**

Isolation of germ layers includes the treatment of embryonic shields with proteolytic enzymes (0.5% trypsin + 2.5% pancreatin in calcium- and magnesium-free Tyrode’s saline at +4°C for 15–30 min) and microsurgery with electrolytically sharpened and polished tungsten needles (Levak-Svajger et al. 1969; Svajger & Levak-Svajger, 1975; see Snow, 1978 for modifications). The isolated pieces are transferred with a specially designed glass pipette under the kidney capsule of syngeneic adult male rats. Teratomas are fixed after 15–30 days and processed for routine histology.

Isolation of germ layers is the crucial step in the procedure because an incomplete separation would render any reasonable interpretation of the results impossible. A clean separation of ‘uncontaminated’ germ layers is facilitated by the existence of enzyme-susceptible basement membranes between them (Pierce, 1966; Adamson & Ayers, 1979) and by the inherent tendency of the ectoderm to rearrange its cytoskeleton and to invaginate during neurulation. The initial detachment of the ectoderm usually occurs spontaneously after treatment with enzymes. Any subsequent surgery can be controlled by careful inspection during manipulation under the operating microscope. The separation of germ layers is only impossible in the region of the primitive streak, where the ectoderm lacks
a basement membrane (see Mitrani, 1982 and Sanders, 1984 for avian embryos) and continuity exists between mesodermal, endodermal and ectodermal cells (Poelmann, 1980a).

**Exogeneous Influences Upon the Graft**

In this experimental system the embryonic ectoderm is subject to atypical exogeneous influences during isolation, grafting procedures and development in the host. These are: (a) interruption of the supply of oxygen and nutrients by uterine blood circulation, (b) influence of new environmental constituents (saline, serum, enzymes), (c) surgical trauma, (d) loss of connection with extraembryonic membranes and of contact with extraembryonic fluids, (e) altered physical (spatial) conditions after transplantation, (f) reduced supply of oxygen and nutrients before full vascularization of the graft, and (g) putative interactive influences from the host tissues. The effects of these factors on the growth, morphogenesis and differentiation of the isolated ectoderm as a renal isograft are poorly understood or completely unknown.

**Isolation and Transfer of the Ectoderm**

Proteolytic enzymes act by digesting the glycoproteins (laminin and fibronectin) of the basement membrane (Zimmermann, Merker & Barrach, 1982). Although the exposure to enzymes is not harmless to the components and the properties of the cell surface (Waymouth, 1974; Maslow, 1976), the cells are subsequently able to restore their basement membrane and other pericellular materials (Osman & Rush, 1981) and to retain a high percentage viability (Dziadek, 1981). In the light of evidence for rapid wound healing in rat embryos (Smedley & Stanisstreet, 1984) and of an efficient regulation of extensive random cell loss in the mouse embryo (Snow & Tam, 1979), the surgical trauma during separation and transfer is not likely to influence the developmental capabilities of the ectoderm.

**Influence of the New Environment**

Prior to isolation the free and the basal surfaces of the embryonic ectoderm are in contact with the amniotic fluid and the basal lamina respectively. After transfer to an ectopic site the early embryonic cells are surrounded by mature tissues of the host. An influence of this new, atypical environment on the grafted ectoderm cannot be absolutely excluded. It is, however, highly improbable that this influence is of a classical inductive type. It has been demonstrated that the basic structure of the renal capsule is essentially the same in various mammalian species. It consists of an outer connective tissue layer, a layer of atypical smooth muscle cells with adrenergic innervation and vascularized loose connective tissue adjacent to the renal parenchyma (Bulger, 1973; Kobayashi, 1978). These structures are unlikely to act as embryonic inducers of differentiation. Differently structured host tissues: the iris of the rabbit (Levak-Švajger & Škreb, 1965), the capsule of the testis (Diwan & Stevens, 1976) and the cheek pouch of the golden hamster
(Damjanov, 1978) also allow a wide range of various tissue differentiation in grafted egg-cylinders. On the other hand, even in a ‘controlled environment’ in vitro non-specific constituents of the substratum and the culture medium can elicit the expression of atypical phenotypes in cultured tissue (differentiation of contracting striated muscle fibers from pituitary cultures, Brunner & Tschank, 1982).

As pointed out previously (Levak-Svajger & Švajger, 1974), the great diversity of differentiation and organotypic tissue associations in teratomas is unlikely to be induced by the host tissues. The presence of tissue derivatives of particular germ layers varies 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 a good deal their initial developmental capacities.

The influence of the host tissues might, however, be of a non-inductive nature. This concerns mainly the proper and quick incorporation and vascularization of grafts which are regularly achieved in a renal site but much less so in an intraocular one (Škreb & Levak, 1960). The influence of the host’s sex hormones (male recipients!) has not been observed since the appropriate target organs (except prostatic rudiments and occasional praeputial glands) have never developed in grafts.

Also in explants of rat embryonic shields cultivated in vitro the quality of tissue differentiation has been shown to vary with respect to the composition and the physical features of the in vitro environment as well as to the frequency of renewal of the culture medium (Škreb & Crnek, 1977, 1980).

**MORPHOGENETIC BEHAVIOUR OF THE EMBRYONIC ECTODERM AS A RENAL ISOGRAFT**

It has been demonstrated that during normal gastrulation in situ of both chick and mouse embryos the primitive ectoderm is subject to temporally and spatially coordinated movements of individual cells in migrating cell streams which use the primitive streak as the passageway to their final destinations (Solursh & Revel, 1978). Approaching the primitive streak the cells of the primitive ectoderm lose their basement membrane (Mitrani, 1982; Sanders, 1984, chick embryo), acquire a bottle-like shape (Tam & Meier, 1982, mouse embryo) and move downwards to incorporate into the definitive endoderm and mesoderm. At the edges of the primitive streak the cells display an active blebbing of the basal surfaces (Sanders, 1984). This is a common feature in embryonic epithelial cells which have lost their basement membranes (Lieb & De Paola, 1981; Osman & Ruch, 1981; Sugrue & Hay, 1981).

In our experiments isolated rat embryonic ectoderm has been transferred under the kidney capsule without the basement membrane which in situ maintained its epithelial structure and regulated the migration of the mesenchyme through the primitive streak (Hay, 1973; Sanders, 1984). This was very probably the major cause of atypical and irregular morphogenetic behaviour of the ectoderm in
isografts, which was described in detail by Švajger et al. (1981). The essential course of events comprises the breaking up of the coherent epithelial structure of the ectoderm and its conversion into an unorganized, mesenchyme-like cell mass. As a secondary event, rosette-like, cystic or tubular structures form out of this mesenchyme. They probably represent the rudiments of the future epithelial structures within the teratomas. Another form of mesenchyme production has been observed in grafts of head-fold-stage embryonic ectoderm: protrusion of cell groups beyond the basal boundary of the epithelium, which is strongly reminiscent of the initial migration of neural crest cells \textit{in situ} (Vermeij-Keers & Poelmann, 1980). Even during normal development of the mouse embryo \textit{in situ}, at the stages prior to neurulation, small focal disruptions of the epithelial organization were observed in the lateral ectoderm of the mouse embryo (i.e. beyond the primitive streak region, Poelmann, 1980a).

Dissociation of the epithelium into amorphous cell aggregates was also observed in renal isografts of the caudal end of the mouse embryo (Tam, 1984), in testicular grafts of 2-cell mouse eggs (Stevens, 1968) and in cultures of chick digestive tract epithelium (Sumiya, 1976). Although considered here as atypical in comparison with normal gastrulation \textit{in situ}, this basic morphogenetic mechanism is at work in various developmental processes in vertebrates: the formation of the neural crest (Vermeij-Keers & Poelmann, 1980) and of the secondary mesenchyme (Hay, 1968), the formation of the tail bud (Bijtel, 1936; Jolly & Féréster-Tadié, 1936; Peter, 1941; Meier & Jacobson, 1982; Nakao & Ischizawa, 1984; Schoenwolf, 1984), and partially in the formation of some cranial peripheral ganglia (Ariëns Kappers, 1941; Verwoerd & van Oostrom, 1979; D'Amico-Martel & Noden, 1983). Even regressive processes in embryogenesis, such as the disappearance of the Müllerian ducts in the male mouse embryos, may involve this mechanism (Trelstad, Hayashi, Hayashi & Donahoe, 1982; Paranko, Pelliniemi & Foidart, 1984). On the other hand, the formation of mesenchyme through an analogoue of the primitive streak is always attained when the early mouse embryo (or the embryonic body) is allowed to develop \textit{in vitro} without disrupting its extra-embryonic cavities and membranes (Wiley & Pedersen, 1977; Wiley, Spindle & Pedersen, 1978; Libbus & Hsu, 1980; Uno, 1982).

The transformation of epithelial cells into mesenchymal ones is a phenomenon of general importance in developmental biology. It can be induced in some embryonic epithelia by appropriate culture conditions (Hay, 1978; Greenberg & Hay, 1982). It is not yet clear whether all epithelia retain an inherent potency to become mesenchymal cells under certain circumstances, nor what constitute the control mechanisms of epithelial and mesenchymal cell shape (Hay, 1973).

The formation of rosette-like, cystic and tubular epithelial structures within the amorphous cell mass apparently occurs during normal morphogenesis of renal tubules and is also comparable with the formation of cavities in aggregates from mixed suspensions of amphibian embryonic cells (Townes & Holtfreter, 1955). Epithelial structures in the posterior end of the mouse embryo such as the secondary neural tube (Criley, 1969; Schoenwolf, 1984) and the tail gut (Švajger,
Kostović-Knežević, Bradamante & Wrischer, 1985) develop by an analogous mechanism. Cystic and tubular transformation of the mesenchyme also occurs in mouse embryos bearing T-locus mutations (Spiegelman & Bennett, 1974; Spiegelman, 1976; Fujimoto & Yanagisawa, 1979).

COMPOSITION OF TERATOMAS

Course of development

In a previous study the course of development of isolated head-fold-stage embryonic ectoderm as a renal isograft was recorded at 2- to 3-day intervals (Levak-Svajger & Svajger, 1979). The above mentioned breaking up into mesenchyme and the formation of epithelial cysts took place during the first three days after transfer. After 5 days the graft enlarged and became vascularized. It contained large amounts of immature neural tissue with areas of massive cell necrosis. The initial formation of the epidermis and myotubes could be observed. The rest of the mesenchyme was undifferentiated. After 7 days the differentiation of the neural tissue and of the epidermis was advanced and the first chondrogenic blastemas appeared. Nine days after transfer the grafts contained abundant neural tissue, striated muscle and cartilage. Ossification started and the epidermis showed the onset of keratinization and of the formation of hair buds. Adipose tissue appeared after 12 days. There was a considerable variation in the size of tumours, even within a single experimental series. In general, it paralleled the duration of development in the host. There was a consistent transition from a discoid shape to massive, tuberous teratomas which only rarely penetrated into the renal parenchyma.

Tissue differentiation

The chaotic arrangement of mature tissues, often associated in a clearly recognizable organotypic combination (Škreb & Švajger, 1975; Švajger, Levak-Švajger, Kostović-Knežević & Bradamante, 1981) conformed to the definition and characterization of benign or mature embryo-derived teratomas (Damjanov & Solter, 1974; Solter, Damjanov & Koprowski, 1975) and of spontaneous benign teratomas observed in man (Willis, 1935, 1962; O’Hare, 1978) and in some strains of mice (Stevens & Hummel, 1957; Stevens, 1967). The tissue composition of teratomas varied according to the initial developmental stage of the ectoderm (see the next section), but in the most favourable cases (the primitive ectoderm grafted for 30 days) the tumours regularly consisted of tissue derivatives of all three definitive germ layers in the classical sense of the germ layer definition:

‘Ectodermal tissues’: skin (epidermis, hairs, sebaceous and mammary glands), neural tissue (brain, neural retina, choroid plexus, ganglia) and others (lentoids, oral cavity, teeth, salivary glands).

‘Endodermal tissues’: foregut-derived epithelia (pharynx, tongue, salivary glands, thyroid, thymus, parathyroid, oesophagus, stomach, respiratory tube,
lung, pancreas), midgut- and hindgut-derived epithelia (small and large intestine, urogenital sinus, prostatic gland).

'Mesodermal tissues': white and brown adipose tissues, cartilage, membrane and endochondral bone, smooth, skeletal and cardiac muscle.

Well-expressed organotypic differentiation and combinations of tissues were comprehensively described and discussed in our previous publications (Škreb & Švajger, 1975; Švajger, Levak-Švajger, Kostović-Knežević & Bradamante, 1981). The endoderm-derived epithelia were outstanding in displaying a wide range of segment-specific features. In general, characteristic associations of tissues, which normally occur during embryogenesis in situ, also occurred in teratomas: pseudo-stratified ciliated columnar epithelium + hyaline cartilage (respiratory tube), stomach and intestinal epithelium with glands + layers of smooth muscle with intramural ganglia (eosinophils and plasmocytes in the lamina propria!), ependyma of the choroid plexus + an ample subependymal vascular network.

With the occasional exception of the neural retina, the mature neural tissue, which histologically mimicked the brain, did not display the cytoarchitectonics typical of any particular segment of the central nervous system. According to observations on the neurulating mouse embryo in situ, the anterior part of the embryonic ectoderm (rostral to the Hensen’s node) gives rise to all segments of the brain (Tamarin & Boyde, 1976; Jacobson & Tam, 1982; Meier & Tam, 1982). There are also structures which never differentiate in teratomas (liver, kidney, adrenal, gonads, germ cells, pituitary, notochord). This could probably be explained by the lack of the delicate topographically and chronologically regulated tissue displacements and interactions which operate during the normal development of these organs in situ. The primordial germ cells are first distinguishable at the posterior end of the late-primitive-streak mouse embryo (Heath, 1978; Tam & Snow, 1981; Snow, 1981; McLaren, 1983; Beddington, 1983a). Their position before and early in gastrulation is not known and very probably they were not included in the primitive ectoderm isolated for grafting in our experiments.

**Incidence of differentiated tissues**

The grafting experiments with isolated germ layers have been based on the presumption that the tissue composition of resulting teratomas reflected the initial developmental potential of the isolated cell population. However, this potential cannot be evaluated only in terms of the incidence of tissues differentiated in grafts. The major characteristics of the experimental embryonic teratomas is their structural polymorphism. The presence of different tissues varies a great deal among grafts of the same embryonic origin (Škreb, Švajger & Levak-Švajger, 1971; Levak-Švajger & Švajger, 1971, 1974). The tissue composition of teratomas is therefore most probably dependent on more factors than the mere developmental capacity of the initial group of embryonic cells. The following factors might be involved. (a) Massive cell necrosis, which exceeds the normal rate of early ectodermal cell death in situ (Poelmann & Vermeij-Keers, 1976) occurs within the immature neural tissue a few days after transfer (Levak-Švajger & Švajger, 1979).
as well as in the mature neural tissue later on (Švajger, Levak-Švajger, Kostović-Knežević & Škreb, 1985). At this time the neuroectoderm grows intensively and this growth is not supported by adequate vascularization. In grafts in which the delay of blood supply is not too long, the developing neural tissue can recover from the initial cell loss and subsequently differentiates into masses of mature neural tissue (the usual feature in large tumours). On the other hand, in some tumours, very probably because of prolonged inadequate vascularization, the neural tissue undergoes further regression and sometimes almost completely disappears. That the amount and quality of tissue differentiation is dependent on an effective graft incorporation and vascularization became evident in experiments with intraocular grafts of the rat egg-cylinders (Škreb & Levak, 1960; Levak-Švajger & Škreb, 1965). (b) Tissues which originate from large presumptive (prospective) areas (neuroectoderm, gut, mesenchymal tissues) differentiate more frequently and in larger amounts than those which in situ develop from restricted areas of the germ layers (teeth, stomach, thymus, thyroid, parathyroid). (c) Some tissues need more time for their final differentiation and therefore appear more frequently in tumours after 30 days (adipose tissue, bone, skeletal muscle, derivatives of the foregut etc.). (d) The phenotypic expression of 'endodermal' differentiation might be masked by the accumulation of intracystic fluid. The resulting distended cysts do not display organotypic epithelial features but are lined by a non-specific, flattened epithelium (Usadel, Rockert, Obert & Schöffling, 1970).

Summing up, the final phenotype expressed in renal isografts of early rat embryonic germ layers results not only from their initial developmental capacities, but also from various, usually ill-defined and uncontrolled events which occur within the interval between the tissue isolation and the fixation of teratomas for histology.

**GERM LAYER ORIGIN OF TISSUES**

The classical view of germ layer origin of various tissues has been modified by the experience that the same tissues can originate from different definitive germ layers (see De Beer, 1947). It is therefore hardly possible to interpret with absolute certainty the complex structure of teratomas in terms of the provenance of different tissues from particular germ layers. This holds especially for some typically 'mesodermal' tissues which may develop from the mesenchyme of both mesodermal and ectodermal (neural crest, tail bud) origin. In this review we use the conventional terms for the germ layers and of their tissue and organ derivatives, while constantly keeping in mind the very restricted developmental value of these terminological designations.

Neural tissue and epidermis are exclusively of ectodermal origin. Neuroectoderm is the first tissue which appears and grows in grafts of the primitive ectoderm (Levak-Švajger & Švajger, 1979). Epidermis develops from undifferentiated epithelial cysts, which form early after grafting (Švajger et al. 1981). The final epidermal phenotype is evident when hair buds appear on the basal side of the
keratinized squamous epithelium. The same type of epithelium (without the skin appendages) may be of endodermal origin. It lines the mucous membranes of the oesophagus and the cardia of the stomach.

Ciliated columnar, pseudostratified epithelium with goblet cells and adjacent glands is the most common epithelial structure in teratomas. It is usually considered as an endodermal tissue, but the nasal cavity which is of ectodermal origin, is also lined by this type of epithelium. Even the large salivary glands seem to be of dual germ-layer origin. The parotid gland is probably of ectodermal origin, while the submandibular and sublingual glands are endodermal derivatives (Hamilton, Boyd & Mossman, 1976). This view is supported by the experience that the parotid gland acts as an integumental derivative and the submandibular gland as a gut derivative in tissue recombination experiments (Kollar, 1983).

The epithelial lining of the intestine is nowadays commonly considered as a derivative of the primitive ectoderm. Not only the experimental evidence (see Rossant & Papaioannou, 1977 and Beddington, 1983b for a review), but also morphological observations indicate this cell lineage (Jolly & Férester-Tadié, 1936; Jurand, 1974; Poelmann, 1981b). A minor contribution of the primitive endoderm to the hindgut was suggested by Jolly & Férester-Tadié (1936). In a recent publication Jackson et al. (1981) argued on the basis of ultrastructural features that the ileum, unlike the rest of the small intestine, is derived from the primitive embryonic endoderm. However the ultrastructure of a cell type is not necessarily dependent on its germ layer origin. As an example, all epithelial cells involved in the transport of water and ions display the same general ultrastructural features, regardless of their embryonic origin (choroid plexus, ciliary body epithelium, stria vascularis of the inner ear, striated ducts of the large salivary glands, convoluted tubules of the kidney). On the other hand, in all our experiments with the primitive ectoderm as a renal isograft, all segments of the primitive gut developed (including the very posterior hindgut derivatives: the large bowel and the urogenital sinus). It seems improbable that only a short segment of the small intestine would have a different origin.

Epithelial organs of mesodermal origin (kidney, adrenal cortex, gonads) never developed in grafts. Mesenchymal tissues, however, appeared regularly and made up a considerable part of teratomas. Except for adipose tissue and cardiac muscle, mesenchymal tissues could also have developed from neural crest cells (Le Lièvre & Le Douarin, 1975; Nakamura, 1982; Nakamura & Ayer-Le Lièvre, 1982). In various experimental systems and in some pathological conditions in situ skeletal muscle has been described as developing from the thymus reticulum (Wekerle, Paterson, Ketelsen & Feldman, 1975; Ketelsen & Wekerle, 1976), Schwann cells (Wallace, 1972; Maden, 1977), pituitary cells (Brunner & Tschank, 1982), glial cells (Lennon, Peterson & Schubert, 1979) and different parts of the central nervous system (for a review see Inestrosa, 1982).

The thymus is perhaps the most intriguing example of unexpected tissue potentialities and differentiation. It develops as an epithelial derivative of the third pharyngeal endodermal pouches, which acquire a mesenchymal (or reticular)
Rat embryonic ectoderm as renal isograft appearance after the invasion of lymphoid stem cells. In the human thymus primordium during the 8th gestational week as well as in the adult human thymus, myoid (myoepithelial) cells have been found, which displayed some morphological features of smooth muscle cells, but showed immunoreactivity with the antisera prepared against striated-muscle-type myosin (Puchtler, Meloan, Brauch & Gropp, 1975; Drenckhahn, Unsicker, Grieser, Schumacher & Gröschel-Stewart, 1978; Gaudecker & Müller-Hermelink, 1980).

One may conclude that, although skeletal muscle is a typical ‘mesodermal’ tissue, all three definitive germ layers bear at least a latent myogenic potential. On the other hand, myoblasts can differentiate into hyaline cartilage when grown on an appropriate substratum (Nathanson & Hay, 1980).

In addition to cardiac muscle, brown adipose tissue seems to be the most typical derivative of the mesoderm. This tissue was the only one to develop in renal grafts of isolated rat embryonic mesoderm (Škreb, Švajger & Levak-Švajger, 1976).

The tail bud, which in all classes of vertebrates appears behind the posterior neuropore, represents a mass of mesenchyme with an extraordinary origin and fate. It forms from the remnants of the primitive streak and by the partial dissociation and migration of neuroectodermal cells of the terminal neural tube. It differentiates into caudal somites and their derivatives, and even back into the neuroepithelium (the ‘secondary neurulation’ and, or, the ‘secondary body formation’, Holmdahl, 1935; Bijtel, 1936; Jolly & Férester-Tadié, 1936; Peter, 1941, 1951; Tam, 1981; Tam, Meier & Jacobson, 1982; Nakao & Ishizawa, 1984; Schoenwolf, 1984; Schoenwolf & Nichols, 1984). Very probably it also contributes cells to the tail gut (Butcher, 1929; Švajger et al. 1985). Thus the tail portions of the neural tube and of the gut develop by aggregation from the diffuse cell mass of the tail bud and their development is strongly reminiscent of the morphogenesis and differentiation of the embryonic ectoderm in renal grafts. It is evident that the capacity of the ectoderm to produce cells with a ‘mesodermal’ fate (the formation of mesenchymal tissues from the neural crest and the tail bud), extends beyond the period of gastrulation. This problem will be further considered in the next section. The data reviewed in this section confirm De Beer’s (1947) view that one and the same tissue type can originate from different definitive germ layers.

RESTRICTION OF DEVELOPMENTAL CAPACITIES OF THE EMBRYONIC ECTODERM DURING GASTRULATION

In the rodent embryo the primitive embryonic ectoderm appears as a distinct cell mass during the blastocyst stage. Its basal surface is lined by the primitive endoderm. During the time interval between the blastocyst stage and the onset of gastrulation, both these primitive (primary) germ layers as a whole (the two-layered embryonic shield) undergo remarkable changes in their geometry (inversion of germ layers), which lead to the formation of the so-called egg-cylinder (see Rossant & Papaioannou, 1977 for review). This unique shape of pregastrulation (preprimitive streak) rat and mouse embryos creates severe
difficulties in both the microsurgical manipulation of the embryo and the interpretation of the isolation and grafting experiments. The latter concerns especially the construction of regionally well-defined fate maps.

We have experimentally approached the problem of diversification of cell lineages during gastrulation by grafting the isolated rat embryo germ layers, excised areas of them and particular germ layer combinations under the kidney capsule of adult male syngeneic animals, and by recording the outcome of differentiation and morphogenesis by the histological analysis of the resulting teratomas. In the following text we will refer to the experimental series designated I-XIV in Table 2.

The embryonic ectoderm as a whole

The primitive embryonic ectoderm of the preprimitive streak stage gives rise to tissue derivatives of all three definitive germ layers: neural tissue, skin, 'mesodermal' tissues and derivatives of the primitive gut (series I, Levak-Švajger & Švajger, 1971).

Embryonic ectoderm isolated together with the initial mesodermal wings at the early primitive streak stage also differentiates into tissue derivatives of all three definitive germ layers (series V, Levak-Švajger & Švajger, 1974).

At the head-fold stage the isolated embryonic ectoderm (regardless of the presence or absence of the primitive streak) differentiates only into 'ectodermal' and 'mesodermal' tissues (series IX, Levak-Švajger & Švajger, 1974).

At all these developmental stages the isolated embryonic endoderm shows no capacity for differentiation as an isograft but becomes completely resorbed shortly after transfer (series IV, VIII, XII, Levak-Švajger & Švajger, 1974).

When at the head-fold stage the definitive endoderm is isolated and grafted together with the adjacent mesoderm, it differentiates into a variety of derivatives of the primitive gut (series X, Levak-Švajger & Švajger, 1974).

At the same developmental stage the isolated embryonic mesoderm differentiates only into the brown adipose tissue (Škreb et al. 1976). However, in combination with the definitive endoderm, it develops into a variety of typical 'mesodermal' tissues (adipose tissue, cartilage, bone, muscle, series X, Levak-Švajger & Švajger, 1974).

These results, confirmed by similar findings in testicular grafts of the mouse primitive ectoderm and endoderm (Diwan & Stevens, 1976) indicate that prior to gastrulation the progenitor cells of all the tissues of the foetal body are located within the primitive embryonic ectoderm. During gastrulation first the endodermal and then the mesodermal cells leave the primitive ectoderm and become displaced to the topographic positions of the respective definitive germ layers. What remains in the topographic position of the primitive ectoderm, becomes the definitive ectoderm of the three-layered embryonic shield (the late primitive streak or the late gastrula stage) which subsequently (at the head-fold stage) undergoes neurulation (see Rossant & Papaioannou, 1977; Beddington, 1983b). From this stage on these three cell sheets have been considered as the definitive
Table 2. *Types of renal isografts reviewed in this paper*

<table>
<thead>
<tr>
<th>Stage (Witschi)*</th>
<th>Stage designation</th>
<th>Rat embryo age (days)</th>
<th>Corresponding age of the mouse embryo (days)</th>
<th>Experimental series (Type of graft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Preprimitive streak (pregastrula)</td>
<td>8</td>
<td>6</td>
<td>I. Whole prim. ectoderm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>II. Transversal halves of the prim. ectoderm</td>
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<td></td>
<td></td>
<td></td>
<td>III. Longitudinal halves of the prim. ectoderm</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV. Prim. endoderm</td>
</tr>
<tr>
<td>12</td>
<td>Early primitive streak (early gastrula)</td>
<td>8.5</td>
<td>6.5</td>
<td>V. Whole prim. ectoderm + mesodermal wings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VI. Transversal halves of the prim. ectoderm + mesodermal wings</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VII. Longitudinal halves of the prim. ectoderm + mesodermal wings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VIII. Prim. endoderm</td>
</tr>
<tr>
<td>13</td>
<td>Head fold (late gastrula, early neurula)</td>
<td>9</td>
<td>7.5</td>
<td>IX. Ectoderm (with or without the primitive streak region)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>X. Endoderm + mesoderm</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>XI. Mesoderm</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XII. Endoderm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XIII. Ant. post. and distal parts of the egg-cylinder</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XIV. Axial and lateral strips of the ectoderm</td>
</tr>
</tbody>
</table>

* See New (1966).
germ layers in the classical topographic, histogenetic and organogenetic sense (Oppenheimer, 1940).

The essential conclusion that all the foetal primordia are descended from the primitive embryonic ectoderm was anticipated in the pioneering experiments of Grobstein (1952) who showed that the early mouse embryonic ectoderm retained its capacity to differentiate into the gut epithelium even as an intraocular graft after 5–7 days of preculturing in vitro. This is also consistent with (1) the morphological observations of the continuity of the Hensen's-node-derived cells with the epithelium of the definitive gut (Jolly & Férester-Tadié, 1936; Jurand, 1974; Poelmann, 1981a,b), and (2) the results obtained by injecting single 5th day mouse primitive ectoderm cells into the 4th day blastocyst. The progeny of the injected cells contributed to tissues and organs throughout the foetus, regardless of their germ layer origin (Gardner & Rossant, 1976, 1979). The rodent embryo shares this basic principle of definitive germ layer formation during gastrulation with the avian embryo, in which it was studied by similar or more direct methods (Nicolet, 1971; Veini & Hara, 1975; Fontaine & Le Douarin, 1977). The analogy of the general developmental principles in these two classes of vertebrates is further suggested by the experimental findings that the embryonic ectoderm of the rabbit is competent to respond to the primary organizer of the chick embryo (Waddington, 1936), and that a secondary neural groove can be induced in the rat embryonic ectoderm by implanted medullary plate material (Törö, 1938; see Waddington, 1957 for a review).

The gradual restriction of developmental capacities of the initially pluripotent primitive embryonic ectoderm and the gradual 'release' of some of these capacities to the newly formed endoderm and mesoderm conform with the claim that "it is only legitimate to speak of the separate germ layers when their segregation from one another is complete" (De Beer, 1958). The segregation of definitive germ layers coincides with the end of gastrulation. However, during the subsequent neurulation, and even later, the cells of the neural crest and of the tail bud originate from the neural ectoderm and differentiate into typical 'mesodermal' and 'endodermal' tissues (see the section entitled Germ Layer Origin of Tissues). Moreover the 'mesectoderm' or 'ectomesenchyme' of the tail bud gives rise to the caudal portion of the neural tube, of the neural crest and of the tail gut (Schoenwolf, 1984; Schoenwolf & Nichols, 1974; Švajger et al. 1985). This corroborates the already old view of the dual origin of the vertebrate body: the primary or indirect development of anterior structures of the body during which the formation of three germ layers precedes the development of the embryonic organ rudiments, and the secondary or direct development of the posterior structures of the body without the intervening formation of germ layers (Holmdahl, 1935; Peter, 1941, 1951).

We may conclude that the embryonic ectoderm never exists as a discrete, definitive germ layer in the classical sense of the word (i.e. as the germ layer with capacities restricted to the formation of the neural tissue and the epidermis). In other words, the three-layered embryonic shield directly transforms only into the
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antior portion of the foetal body. These conclusions are in strict contradiction with the rationale of the classical germ layer theory.

Recently, Lovell-Badge, Evans & Bellairs (1985) investigated the protein synthetic patterns of tissues in the chick embryos from gastrulation and early post-gastrulation stages. This study revealed polypeptides which may be considered as markers for endodermal and mesodermal cell types, but no polypeptides unique for the ectoderm were found. The lack of biosynthetic specialization is in apparent agreement with the capacity of the (neuro)ectoderm to originate various types of tissues even at postgastrulation stages.

Regionalization of the ectoderm and the fate map problem

At both the preprimitive streak and the early primitive streak stages the halves of longitudinally or transversely cut egg-cylinders or isolated primitive ectoderms give rise to tissue derivatives of all three definitive germ layers and there is no indication of a regionally restricted tissue- or organ-forming capacity of the primitive ectoderm (series II, III, VI, VII, Švajger et al. 1981).

At the head-fold stage the ectoderm displays some regionally specific developmental capacities: ectodermal structures of the head (choroid plexus, teeth, vibrissae) develop only from the anterior part of the egg-cylinder (series XIII, Levak-Švajger & Švajger, 1974).

At the head-fold stage the axial and the lateral strips of the ectoderm do not differ in their capacities to differentiate into both neural tissue and epidermis and its derivatives (series XIV, Švajger & Levak-Švajger, 1976).

Attempts to construct on the embryonic ectoderm a map of regions with restricted developmental fates and, or, potencies are relevant to one of the major problems in developmental biology: the mosaic- versus regulative-development dilemma. Recently this has been subject to various experimental approaches in the early postimplantation mouse embryo.

Beddington (1981) introduced the technique of injecting clumps of about 20 [³H]thymidine-labelled embryonic ectoderm cells into the embryonic ectoderm of unlabelled synchronous mouse embryos. The labelled cells were detected in radioautographs of histological sections of host embryos after a 36 h period of cultivation in vitro. Using this method Beddington (1982) carried out a detailed analysis of the developmental fate of embryonic ectoderm cells of the 8th day mouse embryo (late primitive streak). She isolated the donor cells from the anterior, distal and posterior areas of the egg-cylinder and injected them orthotopically and heterotopically into these areas of synchronous hosts. The distribution of injected cells was recorded when the host embryos had attained in vitro the early somite stage. In orthotopic injections the results were as follows: (a) the anterior ectoderm gave rise to predominantly neuroectoderm and surface ectoderm; (b) the distal ectoderm (the area of the Hensen's node) generated definitive gut endoderm, notochord and embryonic mesoderm; (c) the posterior ectoderm (the primitive streak) contributed to the embryonic and extraembryonic mesoderm. In the heterotopic series of injections the distal and posterior
embryonic ectoderm conformed to the colonization patterns characteristic of their new location and their potency always exceeded their developmental fate in situ. The anterior embryonic ectoderm preferentially differentiated into typical ectodermal structures. In all three regions the ectoderm showed the capacity to form 'mesodermal' derivatives. This pluripotency of ectodermal cells conforms to the evidence from other experimental systems, that “the isolated part tends to form a greater variety of structures than when left in place in the embryo” (Harrison, 1933). These results speak against the existence of a rigid mosaicism in embryonic ectoderm, which would be resistant to regulative influences from surrounding cells. So do our results with the grafting of isolated parts of pre-primitive streak and early primitive streak rat embryonic ectoderm (Švajger et al. 1981). It ought to be stressed, however, that the egg-cylinders were transected by randomly oriented cuts only into longitudinal or transverse halves. Even a more elaborate design of isolation of small areas could hardly overcome the difficulties caused by the complex topology of the inverted rodent embryonic shield.

Notwithstanding the above mentioned problems, Snow (1981) isolated circumscribed areas of the whole mouse egg-cylinder (all three germ layers) at the early-, mid- and late-primitive-streak stages, and recorded their development as well as the development of the rest of the mutilated cylinder after a 24 h period of cultivation in vitro. He could demonstrate an autonomous development of isolated pieces and an absence of regeneration or regulation in the rest of the embryonic shield. He interpreted these results as suggesting a mosaic or polyclonal type of development of the early mouse embryo. It is however dubious whether in the topologically enigmatic geometry of the early egg-cylinder one would be able to strictly delineate areas with mutually exclusive and particular developmental fates within any of the constituent germ layers. These operations might also disturb the changes in the relative positions of ectoderm and mesoderm brought about by the differential growth pattern of the embryonic ectoderm (Poelmann, 1980a,b, 1981a,b; Tam & Meier, 1982). The current knowledge on the nature and specificity of epithelial–mesenchymal interactions in development does not yield sufficient information for a convincing interpretation of this experimental system (Lawson, 1974; Yasugi & Mizuno, 1978; Ishizu-Ya-Oka & Mizuno, 1984). As pointed out by Beddington (1982), the fact that the fragments isolated by Snow were composed of all three germ layers, does not allow us to draw any conclusions concerning the mosaic nature of the embryonic ectoderm alone.

Although Beddington’s (1981, 1982) results with embryonic chimaeras are in general agreement with the results of our grafting experiments (Levak-Švajger & Švajger, 1974; Švajger et al. 1981), some differences in the design of the experiments must not be neglected. The grafting of isolated germ layers, whole egg-cylinders or their dissected parts differs from the injection of small groups of cells with regard to the mass and coherence of the ensemble of transferred cells. In the in vitro experiments the results are recorded after 24–36 h, when the cells in question have neither yet reached their final positions nor displayed all their
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devolutional potencies. On the other hand, in grafting experiments a final level of differentiation is attained, but the cell allocation pattern cannot be studied because of the disorganized growth and morphogenesis within the graft.

More recently Beddington (1983a) isolated anterior and posterior slices of both the whole 8th day mouse egg-cylinder and the isolated ectoderm. There were no significant differences between the histogenetic potentials of these fragments in testicular grafts. Both of them generated endodermal structures (epithelium of the primitive gut). This result, as well as the differentiation of the gut epithelium from the orthotopically injected distal ectodermal cells (Beddington, 1981, 1982), could most probably be explained by the relatively early developmental stage of the embryonic shield at the time of manipulation (late primitive streak). At this stage (before the head-fold formation) the embryonic ectoderm has probably not yet exhausted all its prospective endodermal and mesodermal cells, and in the area of Hensen’s node the cells have still retained the tendency to invaginate into the external endoderm layer. At the subsequent head-fold stage gastrulation is completed and the embryonic ectoderm no longer contains prospective endodermal cells (Levak-Švajger & Švajger, 1974). In combination with the mesoderm both the ectoderm and the endoderm display some regionally specific developmental capacities (Švajger & Levak-Švajger, 1974). Whether or not at this stage the ectoderm alone is still capable of forming true mesoderm in renal grafts is uncertain and probably represents a mere semantic question of limited explanatory value in the light of data concerning the developmental capacities of the neural crest and the tail bud (see previous sections).

One might assume that with regard to the overall developmental capacity of the ectoderm and to its regionally restricted potential Beddington’s (1981, 1982, 1983a) experiments in the mouse concern the short developmental interval between the early-primitive-streak stage and the head-fold stage which we investigated in the rat embryo (Levak-Švajger & Švajger, 1974; Švajger & Levak-Švajger, 1974). At the same developmental stage in the mouse embryo, the anterior ectodermal cells injected with horseradish peroxidase contribute primarily to the brain (Lawson, Meneses & Pedersen, 1983).

Tam (1984) transplanted the posterior portion of the primitive-streak-stage (7-5 days) mouse egg-cylinder beneath the kidney capsule. He obtained teratomas composed of tissue derivatives of all three germ layers, but only the presence of hindgut and mesodermal urogenital structures suggested regionally specific differentiation.

The topology and the chronology of cell commitment within the definitive rat embryonic ectoderm is now being subject to analysis in our laboratory. The fact that both the axial and the lateral strips of the head-fold stage ectoderm bear the capacity for neuroectodermal and epidermal differentiation (Švajger & Levak-Švajger, 1976) has to be interpreted with respect to the recent evidence of a strict demarcation of these two ectodermal cell lineages with regard to their cell surface glycoconjugates (Currie, Maylié-Pfenninger & Pfenninger, 1984; Thiéry et al. 1984).
To sum up, the results of our and Beddington’s experiments apparently confirm or, at least, do not contradict the classical experimental evidence that in several classes of the chordate phylum the isolated parts of the early pregastrulation or primitive ectoderm (the blastula ectoderm) are able to regulate to a considerable extent. A restriction of the developmental potential appears during gastrulation and becomes more pronounced (regionalization) at subsequent stages (see Waddington, 1957 and Snow, 1981 for discussion). A fate map comparable to that constructed for the amphibian embryo would only have an explanatory value if it were possible to sharply delineate embryonic cell populations with distinct developmental fates while they are still resident within the same, initial germ layer (the primitive ectoderm). When gastrulation starts the displacement of cell groups brings about new spatial relationships, interactive events and cell commitment occur and are followed by increasing regional restrictions of developmental potencies. A fate or allocation map constructed at the gastrula (primitive streak) stage, as in Snow’s (1981) experiment, can therefore give little or no information in favour or against the mosaic or clonal nature of the early postimplantation development of the rodent embryo.

That even a precisely constructed fate map of prospective (presumptive) areas on the pregastrulation (primitive) ectoderm can fail to reflect the true developmental repertoire of circumscribed groups of embryonic cells, can best be revealed by a careful analysis of data obtained by mapping the amphibian blastoderm. These data represent the basic common knowledge in all textbooks of embryology for more than half a century. The classical schema of the developmental mosaic of the amphibian blastula and early gastrula was constructed on the basis of Vogt’s (1929) vital surface staining and the isolation experiments of Holtfreter (1938). The general conclusion has been that all the future organ rudiments and tissue derivatives of definitive germ layers are represented by circumscribed cell groups on the surface of the late blastula and early gastrula wall. According to Holtfreter, some of these primordia have the capacity for autonomous development (autodifferentiation) when isolated and explanted *in vitro*. However, more recent studies on amphibian gastrulation have revealed a number of details which do not conform to the classical view. (a) The epithelial wall of the blastula and early gastrula is a heterogeneous cell population distributed in superficial and deep positions; (b) these cell groups are subject to independent shifting and displacement during gastrulation (Keller, 1980; Løvtrup, 1983); (c) the cells in different layers of the blastula wall differ in several properties, including prospective significance, inasmuch as “the differences in the properties of cells from the primitive ectoderm inner cell layer in various presumptive regions are less than between the cells of the outer and inner layers in the same presumptive region” (Dettlaff, 1983); (d) when small groups of 15–20 cells from the late blastula wall are isolated and explanted *in vitro*, only the superficial cells form small epidermal vesicles while the deep cells do not differentiate at all (Løvtrup, 1974, 1975, 1983; Landström & Løvtrup, 1979).
It may be concluded from these experimental results that by isolating the ‘ectodermal’ cells of the amphibian blastula and early gastrula the authors have actually isolated cells belonging to at least two definitive germ layers (Nieuwkoop, 1973; Løvtrup, 1975). Only the ectoderm, the notochord and the endoderm appear in the form of presumptive areas on the surface of the blastula. The prospective mesodermal cells are never present on the surface of the pregastrulation embryo. The ‘autodifferentiation’ of prospective mesodermal cells from the surface material occurs as a consequence of interaction of embryonic cells with different prospective germ layer fates. It is therefore interesting to notice that in a most recent publication the early amphibian embryonic cells were defined on the basis of their position in different regions of the gastrula rather than of their germ layer origin (Cleine & Slack, 1985).

Coming back to the problem of terminology, it seems that the German, French and Italian terms for germ layers (Kleimblätter, feuillets embryonnaires, fogliati germinativi = germ leaves) are more appropriate than the English term germ layers, because they denote stretched or extended cell populations without prejudging their monolayer nature.

It has been believed that the amphibian embryo differs from the mammalian one by the early determination of bilateral symmetry (grey crescent), the early commitment of its cells to different developmental pathways if isolated at the blastula stage (Nieuwkoop, 1973) and by the corresponding early susceptibility to exogeneous teratogenic influences (Rugh, 1954). However, it was recently claimed that the vegetal blastomeres of *X. laevis* embryos implanted to another blastula did not become determined before the beginning of gastrulation. At the midblastula stage they contribute progeny to all three definitive germ layers (Heasman, Wylie, Hausen & Smith, 1984). Contrary to previous data from isolation experiments these results suggest that also in the amphibian embryo, at least in some of its regions, the restriction of the developmental potential of the primitive ectoderm may start not earlier than at the early gastrula stage.

**CONCLUDING REMARKS**

The experimental results with ectopic grafts reviewed here apparently reconfirm the view on the common origin of all cells of the foetal body from the primitive embryonic ectoderm, and a gradual topographical and developmental diversification of cell lineages during gastrulation. However, the methodology of histological analysis of experimental teratomas imposes a number of difficulties and limitations in the interpretation of findings in dynamic developmental terms. Besides the fact that the final tissue composition of tumours is largely dependent on the “survival of fittest cells in conditions that are never quite ideal” (Waymouth, 1974), the peculiar morphogenetic behaviour of the primitive ectoderm as a renal graft is quite different from the ordered cell displacement in the living early embryo. Some other problems were stressed before in the classical study of Grobstein (1952): “It seems important to keep in mind that from the (...
differentiative behaviour of such plastic systems it is exceedingly difficult to draw conclusions about the state of determination of their component cells at the time of isolation. In any situation where opportunity is offered for reorganization – particularly the case in transplant, explant and defect experiments – regulation may lead to an increase of cell types or simplification of organization to a decrease of cell types, relative to the prospective significance of the area in question. What is in fact tested in such experiments is the state of determination of the system, not of the component cells.”

The non-specificity of the classical germ layers as to their tissue-forming capacity loads the interpretation with additional problems and demands for a more flexible thinking about cell lineages in early development. The dual origin of most of the ‘mesodermal’ tissues is perhaps the best example. The mesenchyme with the potency to form supportive tissues and muscle originates from three sources: the primitive streak, the neural crest and the caudal neuroectoderm (the tail bud). “There is, however, no essential differences between them, because they originate directly or indirectly from the same ectoderm, and the classical distinction seems to be reducible to the mere difference in stage of mesodermal formation” (Nieuwkoop, 1973). Once again it becomes clear that the germ-layer theory is a morphological concept, and has nothing to do with developmental potencies of embryonic tissues (De Beer, 1947). It is noteworthy that long ago Kölliker (1879, quoted in Oppenheimer, 1940) claimed that “all three germ layers possess the potency and the capacity also for transformation into all tissues, but because of their specific morphological configurations they cannot everywhere manifest this power”.

We may summarize that from the histological analysis of teratomas one cannot draw conclusions on the germ layer origin of tissues but only about the developmental potency of topographically and chronologically defined, isolated regions of the embryo. This seems to restrict the usefulness of the method of ectopic transfer of early mammalian embryonic tissues. However, various experimental approaches on mammalian and avian embryos have given analogous or complementary results which permit us to believe in the existence of common principles of cell lineages in the living embryo and in experimental transplants.

We end with a list of some questions which have arisen from our grafting experiments but cannot yet be answered.

(a) Do particular tissues develop in teratomas from the same precursor cells and by interaction with the same associated cells as they would have in normal development?

(b) How is the neural tissue induced in the absence of a morphologically defined primary organizer? Can we suppose the formation in grafts of an atypical inductive centre, which is able to induce the differentiation of neural tissue but not to organize it morphogenetically?

(c) Do local cell–cell or cell–substrate interactions occur in teratomas, and, if so, do they influence differentiation more than cell lineage, as demonstrated for the mouse extraembryonic endoderm (Hogan & Tilly, 1981)?
(d) Do the transdifferentiation-like phenomena (Yamada, 1977) play any role in the histogenesis within teratomas?

(e) Is the epithelial–mesenchymal conversion of ectodermal grafts a solely morphological phenomenon, or is it correlated with a reorganization of the cellular activity, such as a switching in the expression of cytofilaments from cytokeratins to vimentin (Franke et al. 1982)?

The problem in the broadest sense might perhaps best be revealed by acknowledging the wisdom of one of the old masters of the discipline: “There is always room for fallacy, even when the logical procedure may seem unimpeachable, and no conclusion in embryology is safe if based upon but a single proof. This, to some, may all seem purely formal and of no practical consequence. It is, nevertheless, important to realize that even the language of science is still bound by tradition (...) and it is by no means free from anthropomorphism and relics of our demonology, which are difficult to escape and which may not only lend a false sense of security to our explanations but also may suggest foolish questions that never can be answered” (Harrison, 1933).

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