Int. J. Dev. Biol. 35: 161-176 (1991)

Special Review

Developmental biology of the murine egg cylinder

NIKOLA ŠKREB[1]*, DAVOR SOLTER[2] and IVAN DAMJANOV[3]

[1]Institute of Biology, Faculty of Medicine, University of Zagreb, Republic of Croatia, Yugoslavia, [2]The Wistar Institute, Philadelphia, Pennsylvania, USA and [3]Jefferson Medical College of the Thomas Jefferson University, Philadelphia, Pennsylvania, USA

CONTENTS

***Address for reprints**: Institut of Biology, Faculty of Medicine, University of Zagreb, Salata 3, P.O. Box 166, 41000 Zagreb, Republic of Croatia, Yugoslavia. FAX: 38-41-242.001.

0214-6282/91/$03.00

Printed in Spain

Introduction

Cell-determination and induction of differentiation are two critical issues of both normal and aberrant embryonic development. Beginning with developmentally equipotent and non-committed blastomeres, development proceeds through a series of continuous, but nevertheless discreet phases that are marked on the one hand by acquisition of more and more specialized traits and on the other by a restriction of the originally unlimited development potential.

In the mouse embryo, which is the most extensively studied mammalian model, the interplay of genetic and epigenetic determinants of development is apparent from the earliest stages of embryogenesis. Whereas the blastomeres isolated from 4 and 8 cell embryos are developmentally equipotential (Tarkowski, 1959), positioning of the embryonic cells in the morula (16-32 cell embryo) and compaction specify their developmental fate and whether the cells will give rise to trophectodermal or inner cell mass lineages (reviewed by Maro *et al.*, 1990). However even in the late morulae the outer cells, and likewise the inner ones retain their pluripotency (Rossant and Vijh, 1980) indicating that they are not definitely committed. Formation of the blastocyst and the emergence of two distinct cell populations - the inner cell mass and the trophectoderm leads to the further diversification of cells in the embryo and the appearance of distinct domains that have been extensively characterized morphologically, immunochemically and developmentally (see Gardner 1985; Beddington 1986; Rossant 1987).

Following implantation the embryo forms a highly polarized and complex structure known as the egg cylinder (Fig. 1). The egg cylinder of rodents is equivalent to the gastrula of amphibians and the embryonic plate in human development. It is during these stages of development that the embryo proper definitely separates from the extraembryonic membranes, and the first signs of asymmetry are generated. These are the last stages that contain developmentally totipotent cells, not withstanding the germ cells, whose precursors appear for the first time in the egg cylinder in the form of primordial germ cells (reviewed by Eddie *et al.*, 1981; De Felici and Dolci, 1987). It is arguable whether the events occurring between implantation and gastrulation of the mammalian embryo are any more complex than those in the preimplantation stages of development or in organogenesis. Nevertheless the transformation of developmentally pluripotent cells of the inner cell mass (ICM) of the blastocyst into developmentally restricted germ layers – ectoderm, mesoderm and endoderm – has fascinated us for some time as will be seen from this article.

Our study of early murine embryogenesis began in Zagreb as an attempt to define the developmental potential of cells in the egg cylinder and to characterize various aspects of their differentiation. The work was continued in Philadelphia and Zagreb. In this review, we shall touch upon some of the results gathered in our laboratories concerning murine embryos in early postimplantation stages of development.

The data will be presented as our answers to the question: what did we learn about the developmental potential of the rodent egg cylinder by:

- studying the morphology of the embryo
- transplanting the embryo or its parts to extrauterine sites
- culturing embryos *in vitro*
- and experimenting with the cell lines developed from embryo-derived teratocarcinomas.

Morphogenesis of murine egg cylinder

At the time of implantation the blastocyst of mice and rats comprises two distinct cell populations: the trophectoderm and the inner cell mas (ICM) (Nadijcka and Hillman, 1974). On the blastocelic surface of the inner cell mass a new subset of epithelial cells appears at the end of the fourth day of pregnancy in the mouse and one day later in the rat (Gardner, 1982), developmentally and

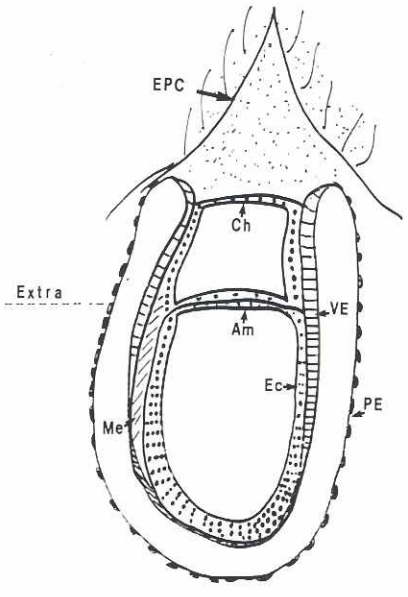


Fig. 1. Diagram of a 7-day mouse egg cylinder. *Ectoplacental cone (EPC) points toward the mesometrial side of the endometrium and is attached to the extraembryonic portion, whereas the embryonic portion is formed by the tip of the egg cylinder facing toward the antimesometrial side. The outermost layer is formed of the parietal endoderm (PE) which is in continuity with the extraembryonic visceral endoderm (VE). The extraembryonic visceral endoderm is in continuity with the embryonic endoderm. Ectoderm (Ec) forms the innermost layer of the egg cylinder. Mesoderm (Me) is interposed in between the ectoderm and mesoderm. Chorionic (Ch) and amniotic folds (Am) are also shown. The dotted line indicates the border between the extraembryonic (Extra) and embryonic portion of the egg cylinder.*

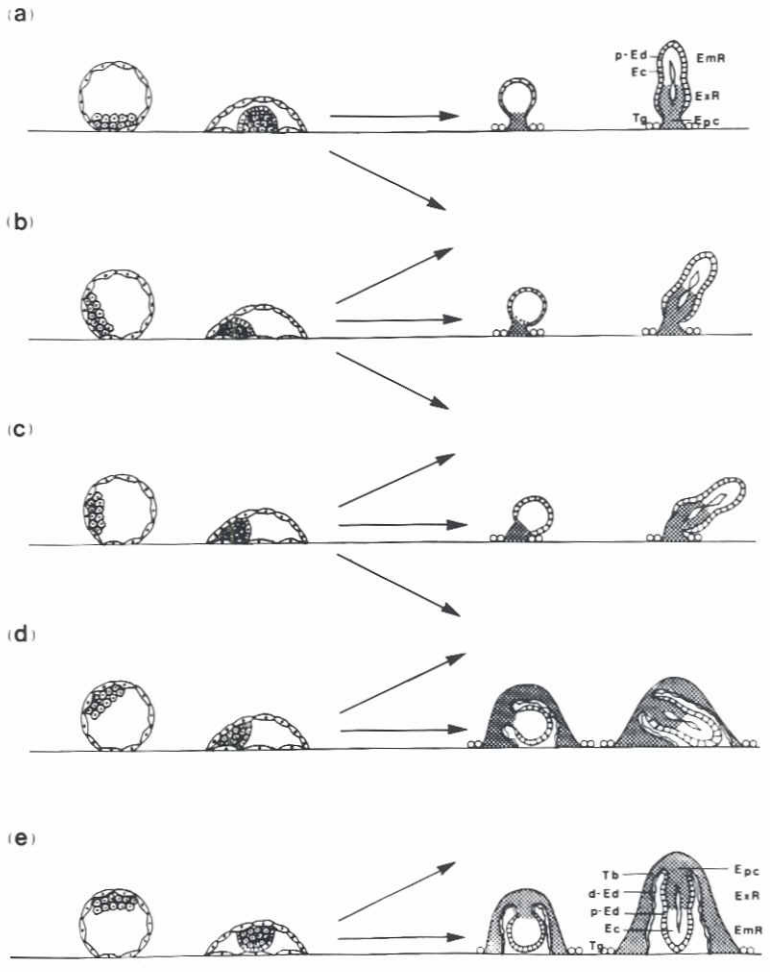


Fig. 2. Diagrammatic presentation of the outgrowth of the mouse egg cylinder *in vitro*. *The embryonic axis formation depends on the positioning of the inner cell mass of the explanted blastocyst. (From Wu* et al., *1981, with permission of the publisher).*

structurally distinct from the remaining ICM cells. It is called the primitive endoderm since it contains the precursors of visceral and parietal extraembryonic endoderm of the yolk sac cells of the choriovitelline placenta (reviewed by Gardner, 1985). The remaining undifferentiated ICM cells form the primitive ectoderm and are considered to be the progenitors of cells and tissues in the embryo proper, the fetus and the adult organism (Gardner and Rossant, 1979).

Implantation of the murine blastocyst is a highly stereotypical event (Schlafke and Enders, 1975). It occurs invariably in antimesometrial crypts formed by the uterine folds primed to become the implantation chamber. After the apposition of the trophectodermal to the endometrial cells, these cells adhere to one another, followed by apoptosis of endometrial cells (Parr *et al.*, 1987). Invasion of trophoblastic cells through the basement membrane into the deeper layers of the endometrium occurs thereafter. However only a part of the murine trophectoderm invades at the site of attachment and the embryo does not dig in completely under the surface layer of the endometrium as in humans (Hertig *et al.*, 1956). The partial intraluminal position of the implanting murine embryo, which is firmly anchored to the antimesometrial endometrium, provides spatial orientation for future development. It also facilitates the proliferation of polar trophectoderm which will form the ectoplacental cone and reach the mesometrial side of the endometrial cavity richly vascularized by the mesometrial uterine arteries.

The implantation of the blastocyst and the formation of the egg cylinder can be studied *in vitro* (Pienkowski *et al.*, 1974; Hsu, 1979). Blastocysts harvested from uteri before implantation can be easily explanted and will hatch from zona pellucida in most culture media. Upon hatching the blastocysts attach to the plastic surface of the culture dishes and continue developing in a manner that is comparable with development *in utero*. Most blastocysts cultured for 3-5 days form egg cylinders, which have all the morphologic features of an equivalent conceptus *in vivo*. Using this approach, we have shown that the formation of the egg cylinder depends critically on the positioning of the ICM at the time of attachment of the blastocyst to the plastic dish (Wu *et al.*, 1981). Hence, the embryonic axis development depends on proper control of the attachment of implanting blastocyst. *In vivo* the attachment of blastocyst to the surface of the uterus occurs always on the mural trophectoderm. *In vitro* the positioning of the blastocyst cannot be controlled and the attachment could thus occur with the mural or polar trophectoderm (Fig. 2). Blastocysts that have attached to the plastic surface with the polar trophectoderm form upward-growing egg cylinders. In others, the axis of the egg cylinder will depend on the initial location of the ICM and the room available for the unobstructed outgrowth of the egg cylinder. Our results show the critical influence of spatial

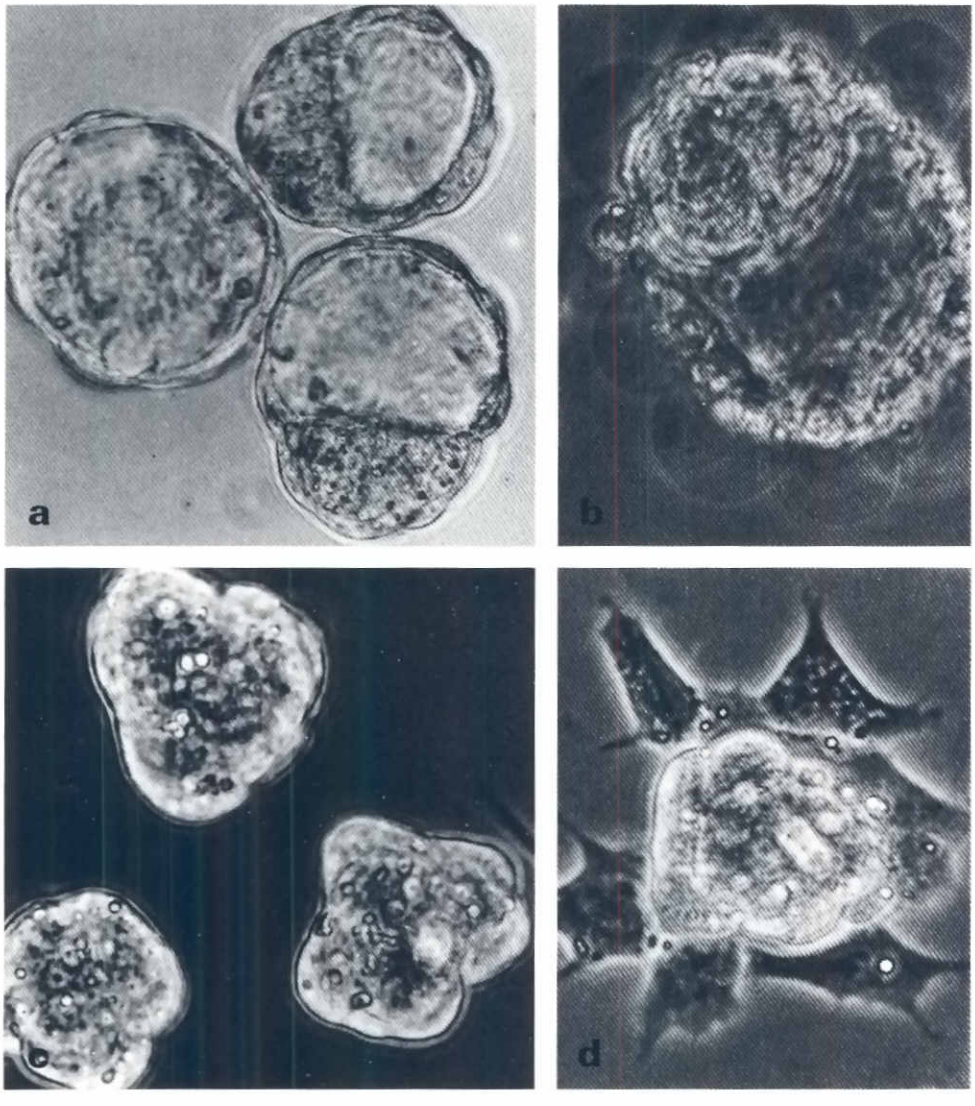


Fig. 3. Immunosurgery performed on mouse blastocysts. *The sequence of early events following exposure of blastocysts to rabbit anti-mouse serum and complement is shown in the upper two panels. In the upper right photograph one can see swelling of the trophoblastic cells and the demarcation of the inner cell mass. In the lower left panel the isolated inner cell masses are denuded of trophectoderm. Right lower panel shows an inner cell mass after 24 hours in culture. (Modified from Solter and Knowles, 1975).*

factors, at least *in vitro*. However it is not clear whether these *in vitro* data faithfully reflect the events occurring *in vivo* (Kirby *et al.*, 1967), and the role of the implantation chamber needs to be explored further.

Most of the egg cylinder is formed from ICM cells of the blastocyst. This was best demonstrated in blastocysts exposed to immunosurgery (Solter and Knowles, 1975), a procedure that selectively removes the outer trophectodermal layer without adversely affecting the ICM (Fig. 3). It is apparent that ICM isolated from early blastocysts are capable of forming trophoblast, suggesting that at least some of the ICM cells are still totipotent (Hogan and Tilly, 1978b). This conclusion is predicated on the very likely assumption that all trophectoderm cells were destroyed by immunosurgery. ICM isolated from fully expanded blastocysts predominantly form structures of increased complexity consisting initially of ectoderm and endoderm with mesodermal components appearing later (Hogan and Tilly, 1978a). It is difficult to make very firm statements about progressive loss of totipotency of ICM cells due to temporal variations among embryos and the absence of precise markers of specific embryonic lineages. It is thus impossible to determine whether structures developing from later ICM contain extraembryonic endoderm (trophectodermal derivative) or not. Nevertheless, analysis of isolated ICMs indicates that the preimplantation period coincides with significant reduction in totipotency of early embryonic cells and that this process is influenced by numerous intrinsic and extrinsic factors (Pedersen, 1986).

Cell populations of the egg cylinder

The 7 1/2-day-old mouse egg cylinder (Solter *et al.*, 1970) or the 8 1/2-day-old rat (Enders and Schlafke, 1967) egg cylinder consists of an embryonic and extraembryonic part (Fig. 1).

Functionally, developmentally and morphologically these two parts of the embryo differ one from another. They are also derived from different cells in the blastocyst: the ectoplacental cone and the extraembryonic ectoderm are derived from the trophectoderm whereas the embryonic part is of ICM origin (Rossant, 1986).

The embryonic part of the egg cylinder consists initially of an

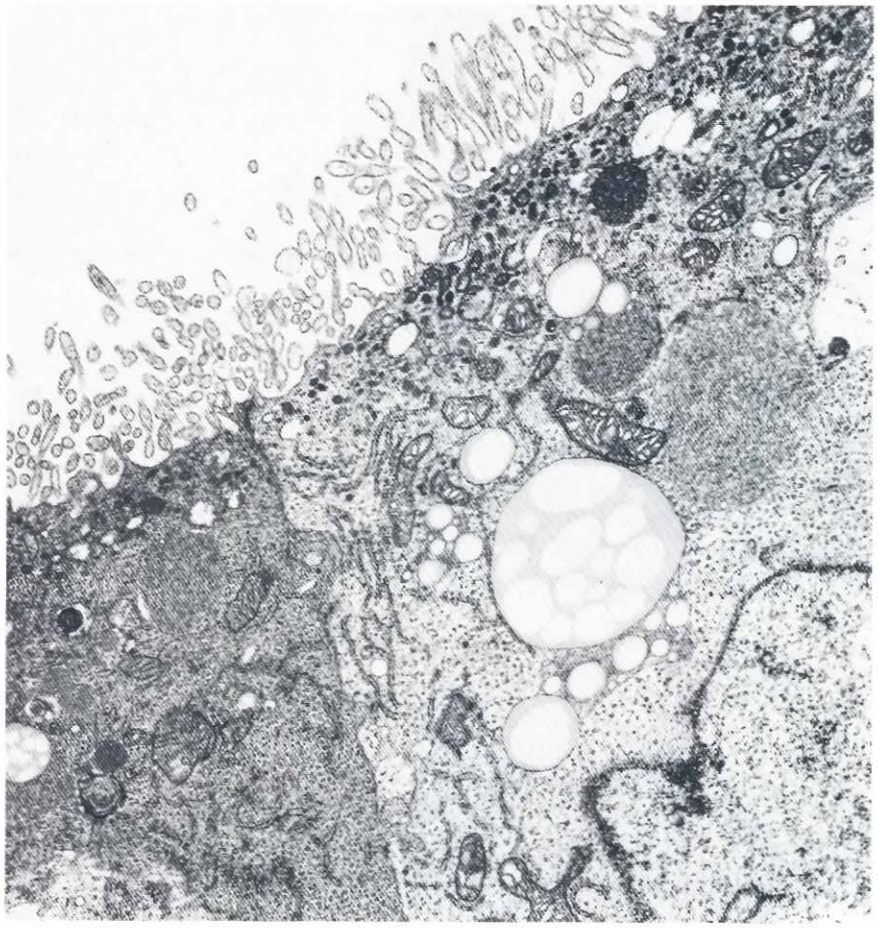


Fig. 4. Electron microscopy of extraembryonic visceral endoderm. *The apical cell surface is covered with numerous slender microvilli and the apical cytoplasm contains numerous lysosomes and absorptive vacuoles. x5200.*

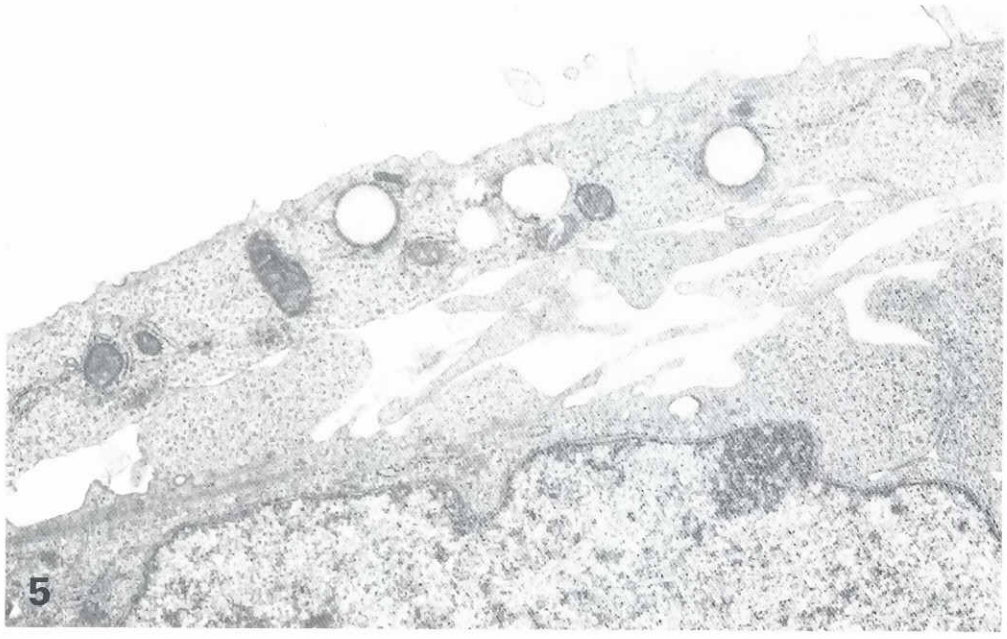
inner ectodermal layer, or epiblast, and an outer endodermal layer (Sobotta, 1911; Huber, 1915). A group of loosely arranged cells appears between the two layers corresponding to mesoderm, the third germinal layer of classical embryology. The developmental potential of these cells has been explored only partially. Although there are no universally accepted developmental maps of the egg cylinder, there is a general consensus that the epiblast or ectoderm represents the only truly pluripotent cell population and that in early stages of egg cylinder development this cell population contains the precursors of all somatic tissues that will develop in the fetus (Gardner, 1985; Rossant, 1987). The restriction of developmental potential and commitment to cell lineages occurs gradually with a considerable amount of cell mixing (Gardner, 1986).

Visceral Endoderm

The entire endoderm of the early egg cylinder is derived from the primitive endoderm, *i.e.*, cells that appear as the first morphologically distinct cell layer on the blastocelic surface of the ICM in the blastocyst (Gardner, 1982 and 1985). The primitive endoderm-derived cells form the extraembryonic endoderm and contribute little if anything to the endoderm on the embryonic part of the egg cylinder. Thus, a 7-day mouse embryo contains two distinct forms of endoderm, which are nevertheless arranged into a continuous outer layer of the egg cylinder.

Functionally and ultrastructurally most differentiated cells in the egg cylinder are the cells forming the visceral endoderm in the extraembryonic part of the embryo (Fig. 4). These cells have a nutrient function (Beck *et al.*, 1967). Ultrastructurally they appear as cuboidal, polarized cells with a well-developed apical surface brush border and numerous absorptive vacuoles in the apical cytoplasm. In contrast, the embryonic endoderm of the 7-day egg cylinder consists of cells that are flattened, show almost no polarization, have few microvilli and almost no absorptive vacuoles and lysosomes (Fig. 5). These differences in the morphology reflect the derivation, developmental potential and fate of cells forming the embryonic and extraembryonic endoderm of the egg cylinder.

Visceral endoderm of the egg cylinder contains mitotic cells (Solter and Skreb, 1968). These dividing cells contribute to the growth of the visceral endoderm itself, but also give rise to parietal endoderm cells (Hogan and Tilly, 1981). It is not known whether the dividing cells represent a bipotential stem cell population similar to primitive endoderm, or whether the visceral endodermal cells are developmentally labile and can transdifferentiate into parietal endoderm (Hogan *et al.*, 1983; Hogan and Newman, 1984). The dividing cells are more prominent in the border zone between the embryonic and extraembryonic endoderm. Ultrastructurally the dividing cells do not contain the full complement of organelles typically seen in the extraembryonic visceral endoderm and thus appear less differentiated (Fig. 6). This is more consistent with a stem cell population theory than transdifferentiation, but the latter



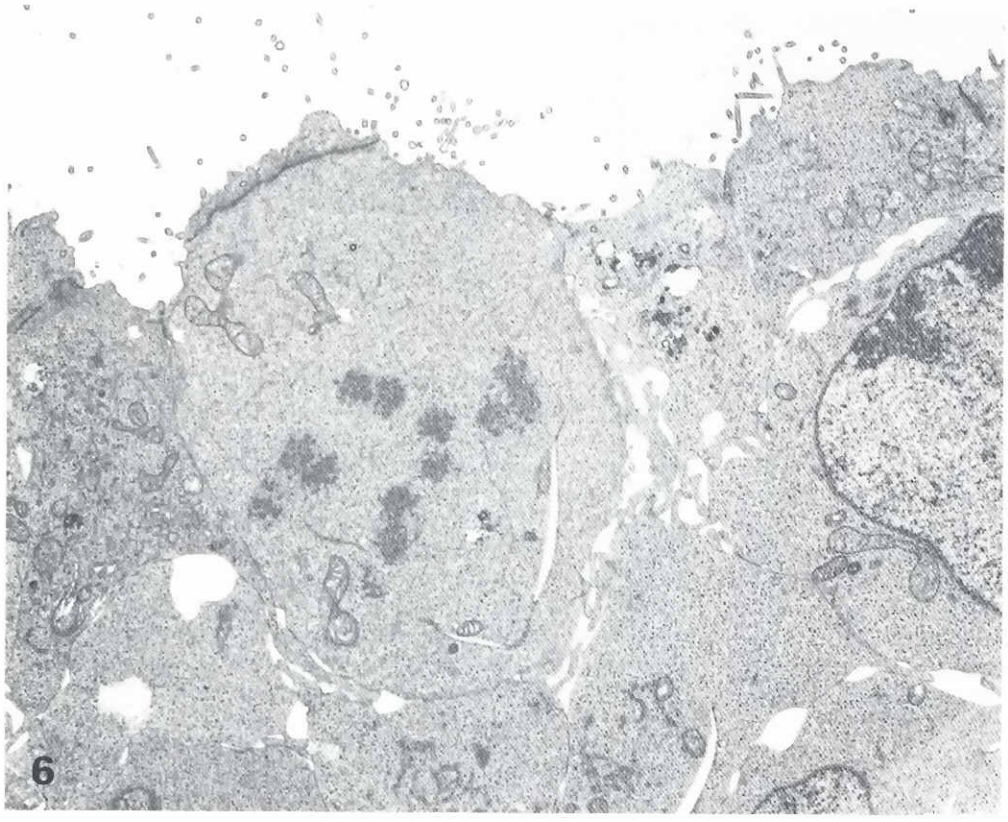


Fig. 5. Electron microscopy of embryonic visceral endoderm. *The flattened cell has only a few sparse microvilli on its surface. The cytoplasm contains few organelles and no absorptive vacuoles. There are a few lipid droplets and mitochondria. x16800.*

Fig. 6. Electron microscopy of a dividing visceral endoderm cell. *The cell contains few organelles. x7200.*

explanation cannot be excluded with certainty, especially in view of the experimental data with mouse embryonal carcinoma cells (Hogan *et al.*, 1983).

The primary function of the extraembryonic endoderm is the uptake of nutrients and other substances, such as immunoglobulins passed from the mother to the embryo. In order to perform this absorptive function the endodermal cells are endowed with hydrolytic enzymes such as acid phosphatase and esterase (Rode *et al.*, 1968; Solter *et al.*, 1973). In early egg cylinders the entire endoderm contains abundant acid phosphatase and esterase positive lysosomes. In the later stages, after the appearance of the mesoderm, almost no acid phosphatase can be seen in the

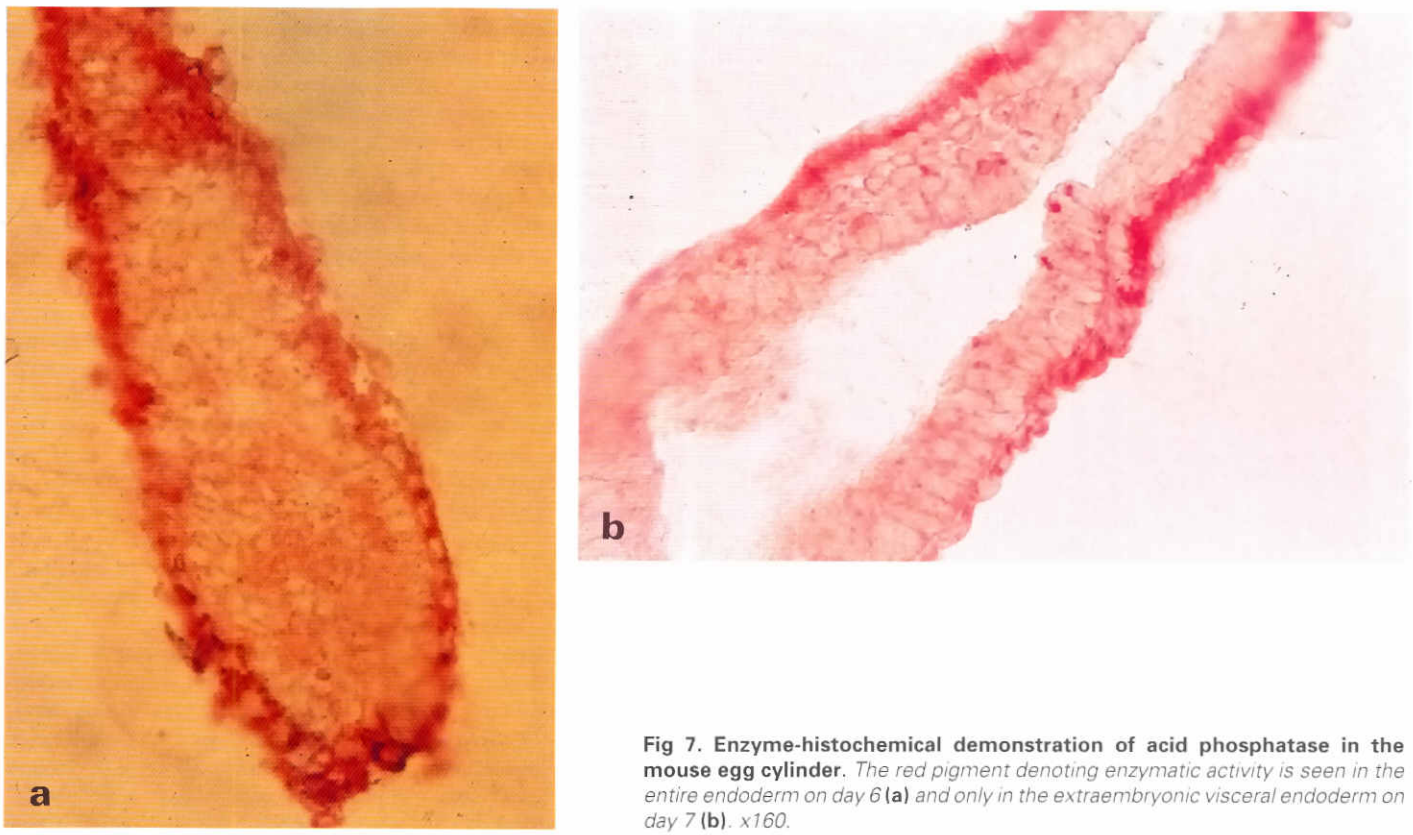


Fig 7. Enzyme-histochemical demonstration of acid phosphatase in the mouse egg cylinder. *The red pigment denoting enzymatic activity is seen in the entire endoderm on day 6* **(a)** *and only in the extraembryonic visceral endoderm on day 7* **(b)**. *x160.*

embryonic endoderm while the extraembryonic endoderm remains strongly positive (Fig. 7). These acid phosphatase negative cells most likely represent a new distinct population of cells formed from the primitive ectoderm (Lawson and Pedersen, 1987).

Immunohistochemical (Fox *et al.*, 1981, 1984; Sato *et al.*, 1985) and lectin histochemical (Wu *et al.*, 1983) studies reviewed by Muramatsu (1988), Solter and Knowles (1986) and Fenderson *et al.* (1990) have illustrated the biochemical complexity of the cell surface molecules expressed on the visceral endoderm. However these studies have not solved the problems pertaining to the exact derivation of these cells (Pedersen *et al.*, 1977), nor have they elucidated the developmental potential of various embryonic cells. In brief some of the cell surface markers are common to primitive and visceral endoderm, others are expressed only on the visceral endoderm, and still others are limited to the extraembryonic visceral endoderm only (Fig. 8). It is of interest to note that certain markers are expressed on both visceral endoderm and ectoderm, but none of the surface markers are common to the visceral and the parietal endoderm. Thus, although the extraembryonic visceral and parietal endoderm have common precursors and although they may even share a common derivation from the primitive endoderm, the two cell lineages are immunochemically and morphologically distinct one each other.

Visceral endodermal cells provide nutrients to other embryonic cells in the egg cylinder. It is not obvious whether this support function is limited to a transfer of absorbed maternal nutrients or whether it also includes paracrine effects mediated through growth factors and metabolites produced by these cells. Antibodies to the apical cell surface components, presumably involved in absorption of maternally-derived metabolites, interfere with normal development and produce malformations (Jensen *et al.*, 1989), further emphasizing the pivotal role of visceral endoderm in the development of the egg cylinder. The nature of the interaction between the visceral endoderm and the other cells, the nature of signals transmitted from the outer to inner layers of the egg cylinder and the various growth factors secreted by visceral endoderm remain to be elucidated. Furthermore, the role of various cellular protooncogenes, and growth factor receptors, some of which are prominently expressed in the visceral endoderm (Adamson, 1986) remains unknown.

The developmental potential of the cells forming the visceral endoderm has been explored by transplanting the egg cylinder or isolated germ layers to extrauterine sites (reviewed by Svajger *et al.*, 1986); by heterotopic grafting of isolated segments from one egg cylinder to another (reviewed by Beddington, 1986); and by injecting exogenous markers into single embryonic cells (Lawson *et al.*, 1986; Lawson and Pedersen, 1987). These studies show that the extraembryonic visceral endoderm consists almost exclusively of progenitors of the visceral layer of the yolk sac and that the embryonic endoderm gives rise to the embryonic gut (Levak-Svajger and Svajger, 1971, 1974; Lawson *et al.*, 1986).

Visceral endoderm cells synthesize proteins for export (reviewed by Adamson, 1986). Some of these are incorporated into extracellular matrix and others are released into the body fluids. Among these the best characterized soluble secretory products is alpha-fetoprotein (AFP), a serum glycoprotein of 75,000 daltons coded in mouse by a single gene on chromosome 5. In seven day embryos AFP is synthesized by the embryonic visceral endoderm. The extraembryonic visceral endoderm does not produce AFP, presumably due to an

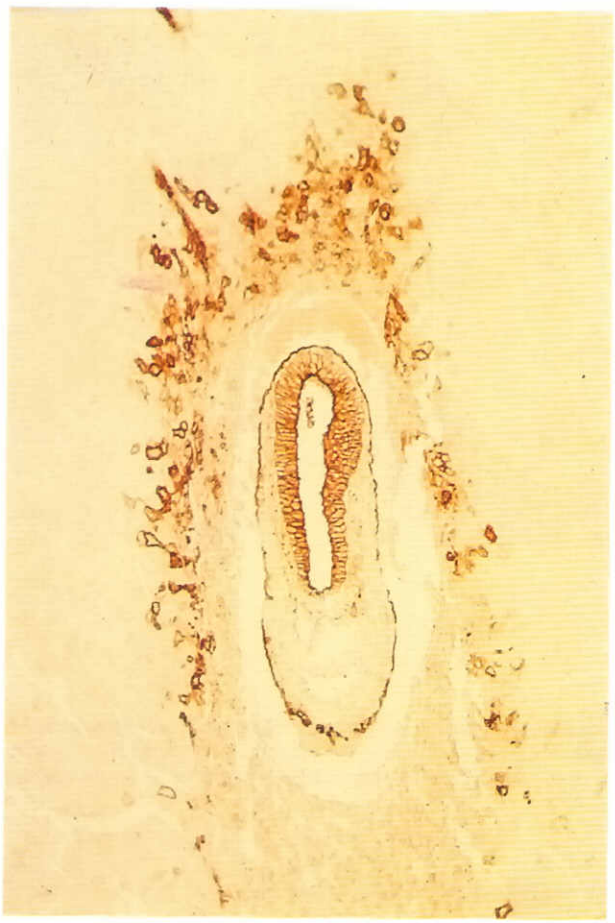


Fig 8. Immunoperoxidase stain demonstrating the localization of SSEA-1 in the embryonic ectoderm and the visceral endoderm. *Trophoblastic cells surrounding the embryo are also immunoreactive. x90.*

inhibitory effect of the extraembryonic ectoderm (Dziadek, 1978). In contrast to the extraembryonic visceral endoderm, which has a latent potential to produce AFP when cultured without adjacent ectoderm (Dziadek, 1978), the parietal endoderm does not produce AFP under any circumstances. Thus AFP could be used as a reliable cell lineage marker of visceral endoderm.

Other serum proteins produced by the visceral endoderm include transferrin, apolipoprotein A1, alpha-1-antitrypsin, metallothionein (Adamson, 1986). In adult rodents these serum proteins are derived from the liver indicating that the visceral endoderm has some of the properties and functions in common with the liver. However, in contrast to the adult liver, visceral endoderm exhibits a very low level of activity of the albumin gene.

Visceral endoderm produces several matrix proteins such as laminin and collagen type IV (Adamson, 1986). These proteins are incorporated into the basement membrane separating the endoderm from ectoderm (Fig. 9). Ultrastructurally this basement membrane appears as a thin condensation of extracellular matrix in the form of a lamina densa, but without a distinct lamina rara (Fig. 10). Thus, it differs from the much thicker Reichert's membrane formed by the parietal endoderm and the parietal yolk sac (PYS). Immunochemically the two basement membranes also differ from each other (Wan *et al.*, 1984; Wewer *et al.*, 1987) and are, at least with regard to their immunoreactivity with monoclonal antibodies to laminin, distinct from the basement membranes in the adult tissues (Damjanov, 1990). It has also been shown immunohistochemically that these embryonic basement membranes change during development, the changing structure and composition probably reflecting their changing function during morphogenesis (Wan *et al.*, 1984).

Parietal endoderm

Parietal endoderm is composed of a unique cell population that arises from the primitive endoderm soon after the implantation of the blastocyst (reviewed by Hogan *et al.*, 1983). In contrast to visceral endodermal cells, which are interconnected one with another with desmosomes and adhesion proteins, such as uvomorulin (Damjanov *et al.*, 1986) the parietal endoderm, like its immediate descendant, the parietal yolk sac (PYS), consists of migratory cells that do not form firm intercellular junctions. Nevertheless these cells contain a cytoskeleton composed of keratin polypeptides, which indicates their epithelial, rather than mesenchymal nature (Lane *et al.*, 1983).

Parietal endoderm consists of cells that show low mitotic activity (Hogan *et al.*, 1983). Thus, in order to grow, the parietal endoderm recruits cells from the dividing cell pool in the visceral endoderm. The nature of this acquisition is subject to speculation and could occur either from a pool of undifferentiated stem cells or through transdifferentiated visceral endodermal cells (Hogan *et al.*, 1983).

The major, if not the primary, function of the parietal endodermal cells is to form and maintain the Reichert's membrane (Pierce *et al.*, 1962). Reichert's membrane of the egg cylinder or older embryos contains typical basement membrane components such as laminin, entactin, collagen type IV (Martínez-Hernández and Amenta, 1983). Ultrastructurally the basement membrane appears layered and contains several laminae rarae and laminae densae (Fig. 11).

Fibronectin is present in the Reichert's membrane from the early stages of its development (Wartiovaara *et al.*, 1979). However in embryos cultured *in vitro*, fibronectin is found only in the basement membrane produced by the primitive and visceral endoderm (Damjanov *et al.*, 1990). Since parietal endoderm does not secrete fibronectin, it appears that the immunochemically detectable fibronectin in the Reichert's membrane represents soluble fibronectin that was incorporated into the basement membrane during its passage across the feto-maternal barrier (Amenta *et al.*, 1983).

Osteonectin, also known as SPARC (Mason *et al.*, 1986) is another major secretory product of the parietal endoderm. However it is not readily detectable in the Reichert's membrane (Damjanov *et al.*, 1990) indicating that it is most likely discharged in a soluble form into the body fluids. From this one could conclude that the function of parietal endodermal cells is not restricted to basement membrane production and that a detailed search for secretory proteins could reveal a more complex participation of these cells in the maintenance and nurturing of the embryo and the integrity of materno-embryonic barrier.

The developmental potential of parietal endodermal cells seems to be limited to extraembryonic cell lineages within which it appears to contribute only to the formation of the parietal yolk sac (Hogan *et al.*, 1983). The study of the histogenetic potential of the parietal endoderm has been hampered by the low mitotic rate and the inability of these cells to grow *in vitro* or *in vivo* upon transplantation

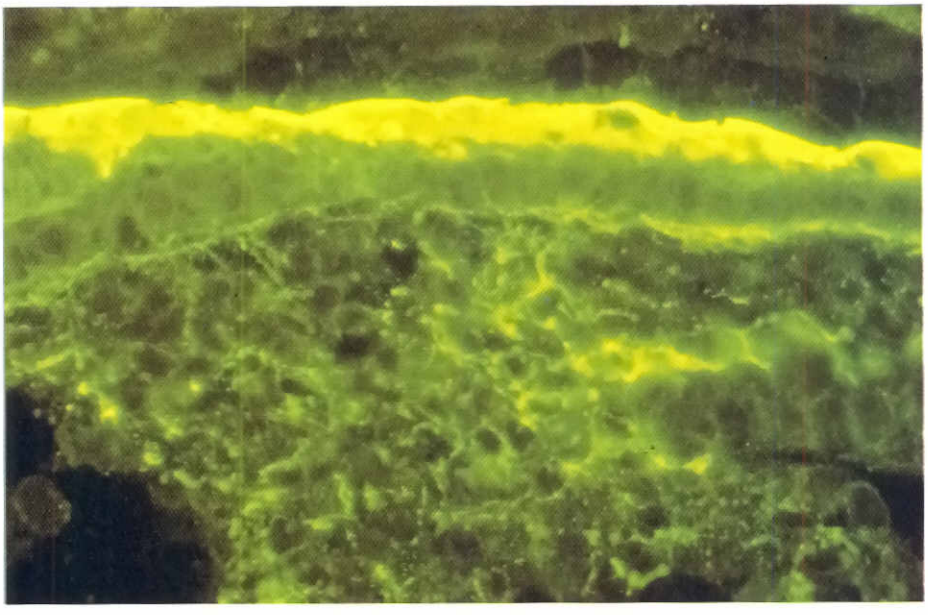


Fig 9. Immunofluorescent microscopic demonstration of laminin in the embryonic basement membrane. *x160. The thick upper line is the Reichert's membrane. The thin line is the basement membrane between the visceral endoderm and ectoderm. Note that there is also intercellular laminin between the ectodermal cells. x240.*

to extrauterine sites. We were unable to obtain any tissues from parietal endodermal cells explanted underneath the kidney capsule. In contrast, teratomas and PYS carcinomas can be readily produced from midgestational yolk sac left *in utero* following fetectomy (Sobis *et al.*, 1982). Thus, either the placental yolk sac contains some pluripotent stem cells or these stem cells originate through transdifferentiation from the parietal or visceral endodermal layer of the yolk sac. Likewise, the small nodules composed of parietal endodermal cells that were produced by transplanting the extraembryonic portion of the 7-day egg cylinder (Solter and Damjanov, 1973) could be descendants of undifferentiated precursors of the parietal endoderm within the visceral endoderm or from transdifferentiated visceral endodermal cells.

Ectoderm

The ectodermal layer of the egg cylinder (also known as epiblast) is composed of cells that are the direct descendants of the ICM cells remaining after the segregation of the primitive endoderm (Gardner, 1985). These cells are considered developmentally pluripotent and are, at least in the early stage of development, precursors of essentially all fetal tissues. In the primitive streak or somewhat older egg cylinders, the ectoderm consists of specific regions that contain developmentally-determined cells (Beddington, 1981, 1982, 1983).

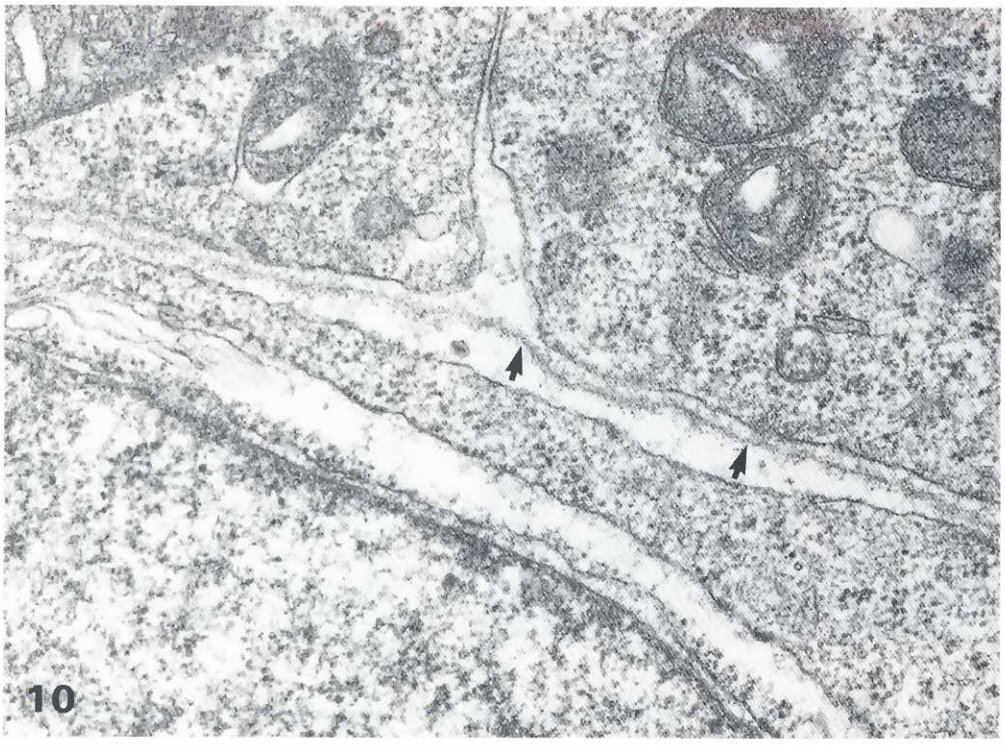
Ultrastructurally the ectodermal cells of the egg cylinder appear undifferentiated (Solter *et al.*, 1970). The nucleus of these cells contains finely dispersed euchromatin and the cytoplasm contains few organelles except for free ribosomes (Fig. 12). In the 7-day egg cylinder the cells of the embryonic and extraembryonic ectoderm are arranged into a well-formed epithelial layer of firmly interconnected cells (Fig. 12).

Ectoderm of the embryonic and the extraembryonic portion of the early egg cylinder displays strong activity of alkaline phosphatase (Rode *et al.*, 1968; Solter *et al.*, 1972, 1973), which is the best enzymatic marker for this cell layer (Fig. 13). Immunohistochemically, the ICM and ectoderm of the egg cylinder express a stage-specific embryonic antigen (SSEA-1) recognizable with a monoclonal antibody (Solter and Knowles, 1978). The epitope of SSEA-1 was defined as fucosylated N-acetyl lactosamine, equivalent to the human blood antigen Le^x (X-hapten) (reviewed by Solter and Knowles, 1986; Fenderson *et al.*, 1990). The antibody to the monomeric X-hapten reacts with the entire ectoderm (Fig. 8), whereas the antibody to the dimeric X reacts only with the luminal surface of ectodermal cells lining the proamniotic cavity (Fenderson *et al.*, 1986). Antibody to I blood group antigen reacts with extraembryonic ectoderm, but not with the embryonic ectoderm, pointing to the differences between ectoderm in the two parts of the egg cylinder (Fenderson *et al.*, 1988). Developmentally, the extraembryonic ectoderm also differs from the ectoderm in the embryonic portion of the egg cylinder (Rossant and Ofer, 1977).

Mesoderm

Mesodermal cells emerge from the primitive streak ectoderm as a group of dissociated cells (Sobotta, 1911). Ultrastructurally the mesodermal cells resemble their progenitors in the ectodermal layers and appear undifferentiated (Solter *et al.*, 1970; Batten and Harr, 1979; Fig. 14). However due to their loose association these cells do not form desmosomes or complex intercellular junctions. In contrast to ectoderm and endoderm, mesodermal cells do not express uvomorulin, a calcium-dependent cell-to-cell adhesion molecule (Damjanov *et al.*, 1986). The cytoskeleton of mesodermal cells contains vimentin, the typical intermediate filament protein of mesenchymal cells in general (Franke *et al.*, 1982). The motility of these mesodermal cells has been documented cinematographically (Nakatsuji *et al.*, 1986).

The monoclonal antibody to blood group antigen I is a useful reagent for detecting mesodermal cells (Fenderson *et al.*, 1988). The first I antigen-positive cells appear in the 6.5 embryos within the posterior amniotic fold. However I antigen appears only temporarily on mesodermal cells and is lost gradually during the neurulation. This is probably due to a general loss of endo-beta-galactosidase-susceptible lactosaminoglycans from mouse embryos upon transition



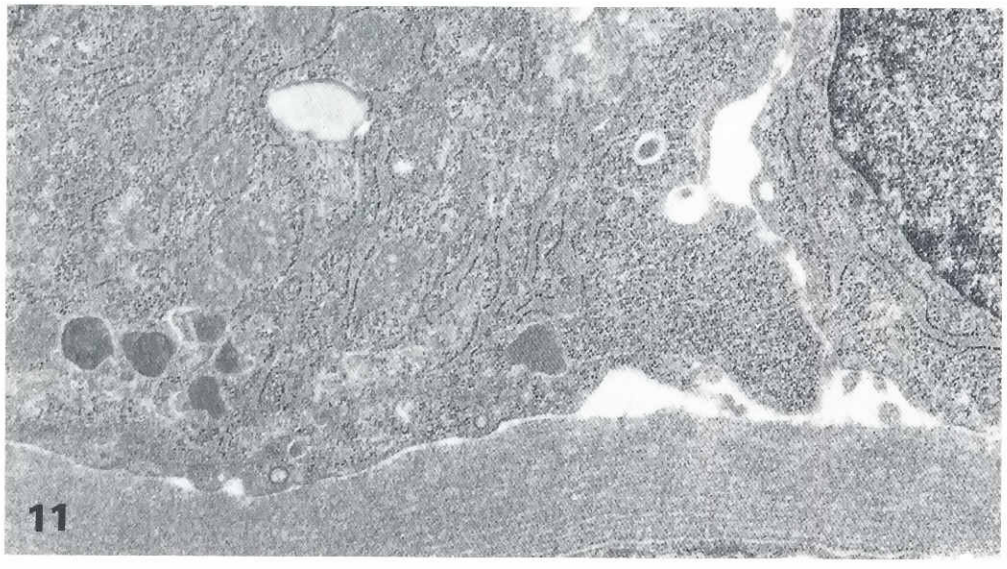


Fig. 10. Electron microscopy of the basement membrane between the embryonic ectoderm and endoderm. *x47500.*

Fig. 11. Electron microscopy of the rat Reichert's membrane shows multiple layers. *The adjacent parietal endoderm cell has well-developed rough endoplasmic reticulum. x45000.*

of the egg cylinder into the organogenetic phases of development (Muramatsu, 1988).

The nature of signals that lead to the formation of mesoderm is poorly understood. Theoretically, as in lower vertebrates which were studied in greater detail, the induction could be «instructive» or «permissive»; based on cell-to-cell interaction or controlled by growth factors and inducers/inhibitors of differentiation (Jacobson and Sater, 1988). Transforming growth factor beta, basic fibroblast growth factor and other morphogens play an important role in the induction of mesoderm in amphibians (Smith, 1989) and their role in the formation of mesoderm of rodents deserves closer scrutiny. It is however important to note that the recruitment of mesodermal cells from ectoderm is a continuous process and that it occurs in different forms (Tam, 1989). Finally it is obvious that at each point of development mesoderm comprises heterogeneous cell populations which constantly undergo a considerable amount of cell mixing (Tam and Beddington, 1987).

The developmental fate of mesodermal cells in the primitive streak embryo has not been fully explored. One of the reasons is the limited growth potential of isolated mesoderm transplanted to extrauterine sites where it invariably differentiates into brown adipose tissue unless it is transplanted together with the endoderm (Levak-Svajger and Svajger, 1974).

Primary ectoderm-derived mesoderm gives rise to the so-called

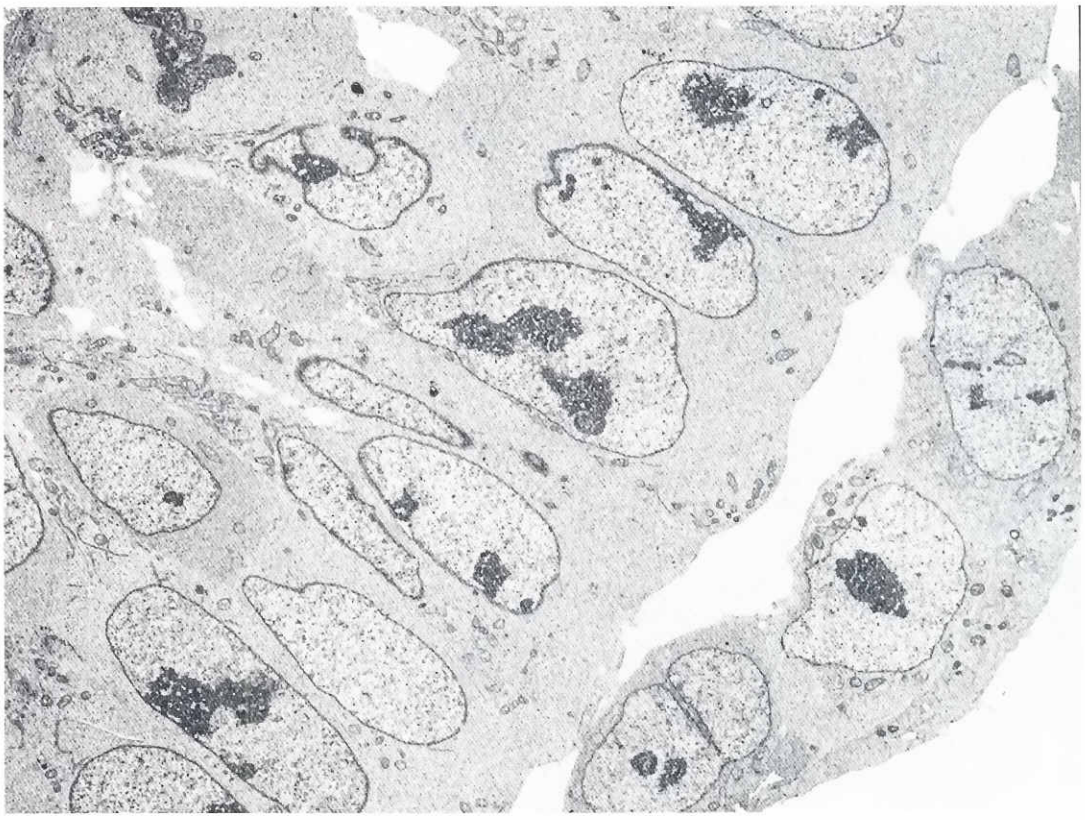


Fig. 12. Electron microscopy of the embryonic part of the 7-day mouse egg cylinder *shows aligned outer ectodermal cells separated from the visceral endoderm by an intercellular space. The innermost ectodermal cells are loosely arranged. x3500.*

intermediate mesoderm in the somites. Primary mesodermal cells partially disperse to generate the secondary mesoderm (mesenchyme) from which are formed the final mesodermal derivatives such as sclerotomes, dermatomes, and myotomes (Svajger *et al.*, 1986). Mesoderm also participates in the formation of the extraembryonic structures, most notably the allantois (Ellington, 1985). The similarities and differences between the primary mesoderm in the egg cylinder and the various secondary mesodermal cells have not been explored.

Embryo-derived teratocarcinoma

We have studied the developmental potential of the rat egg cylinder by transplanting it to extrauterine sites (Skreb *et al.*, 1976) or by culturing it *in vitro* (Skreb and Svajger 1973). Serendipitously, while transplanting seven-day mouse egg cylinders underneath the kidney capsule of syngeneic adult mice, we discovered that some of the grafts grow rapidly, attain an enormous size and biologically behave like malignant tumors (Solter *et al.*, 1970; Stevens, 1970). Histologically these embryo-derived tumors had the appearance of teratocarcinomas and contained undifferentiated stem cells, equivalent to embryonal carcinoma (EC) cells (Stevens, 1967; Damjanov *et al.*, 1971a,b). In a series of experiments reviewed by Damjanov and Solter (1974) and Solter and Damjanov (1979), we showed that the EC cells in embryo-derived teratocarcinomas represent «malignant» equivalents of ectodermal cells in the egg cylinder. This was confirmed by Diwan and Stevens (1976), who were able to produce histologically identical tumors from isolated ectoderm of 6-day mouse egg cylinders. The embryonic nature of EC cells and the reversible nature of their «malignancy» was demonstrated by injecting EC cells into the normal blastocyst (Brinster, 1974), in which these cells become incorporated into the ICM and contribute to essentially all somatic tissues developing from that embryo (reviewed by Mintz and Fleischman, 1981).

Embryonal carcinoma cells derived from mouse teratocarcinomas have been extensively used as replicas of ectodermal embryonic cells from the early stages of murine development (Martin, 1981). The initial method for generating EC cells from embryos transplanted to extrauterine sites has been superseded by a more direct cloning of embryonic stem (ES) cells from the ICM cultured *in vitro* (Evans and Kaufman, 1981; Martin, 1981). The recent discovery that a leukemia differentiation-inducing factor promotes the growth of undifferentiated embryonic cells (Smith *et al.*, 1988) has considerably increased the success rate of ES cell production *in vitro*.

In contrast to blastocysts which can give rise to ES cells *in vitro*, explanted egg-cylinders all differentiate into non-proliferating tissues (Skreb and Svajger, 1973; Skreb and Crnek 1980). Comparable results have been obtained in serum-free and protein-free media which allow the survival of explants for an extended period of time (Skreb and Bulic, 1987).

The effects of various sera that induce or modify growth and differentiation have been examined (Skreb *et al.*, 1983). One can conclude that the embryonic cells of the egg cylinder are developmentally pluripotent. Certain pathways of differentiation, like formation of neural tissue and lentoids (Bulic-Jakus *et al.*, 1990), occur more in serum or transferrin supplemented media. However, it has so far not been possible to obtain undifferentiated embryonic cells from egg cylinders grown *in vitro*.

Although the recent technical improvements for derivation and

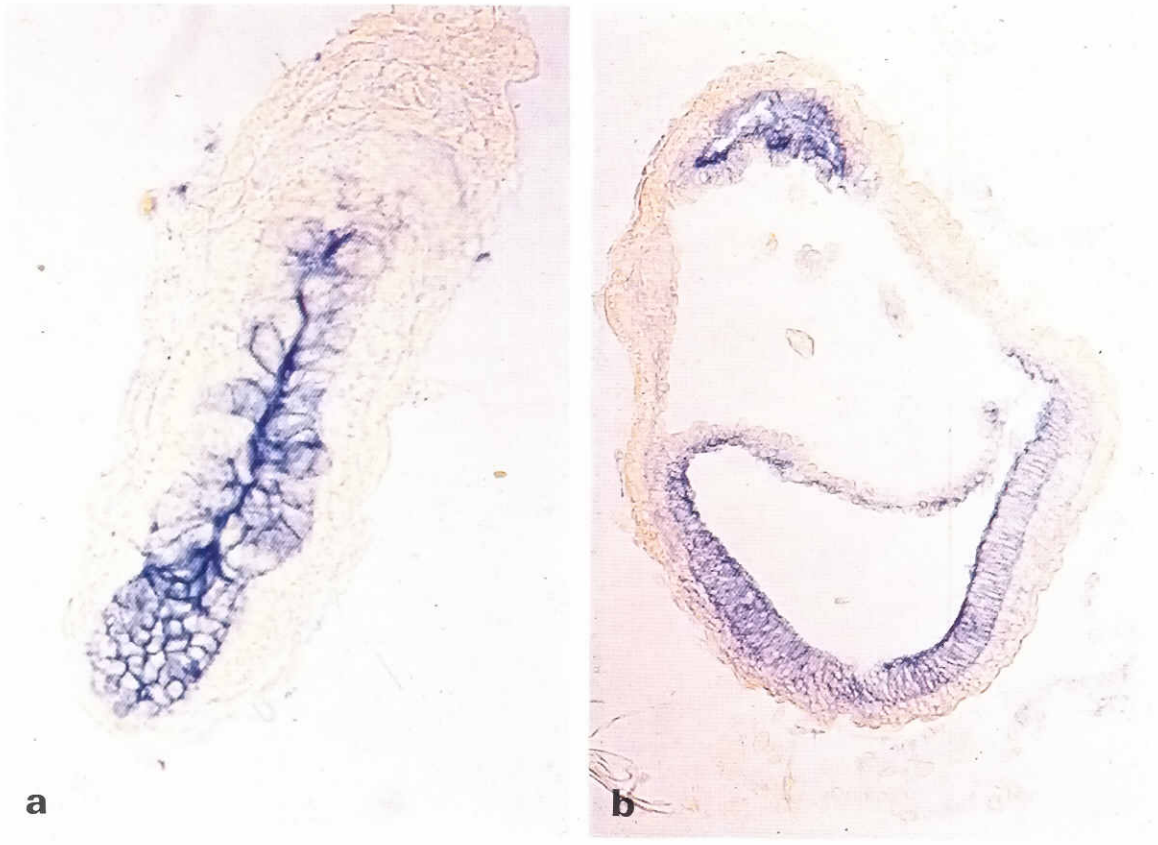


Fig. 13. Enzyme-histochemical demonstration of the alkaline phosphatase in an early (a) and a later stage egg cylinder (b). *Activity of the alkaline phosphatase blue can be demonstrated only in the ectodermal cell layer. x160.*

cloning of embryonic stem cells make the embryo grafting technique obsolete, the experiments based on syngeneic egg cylinder transplantation have nevertheless contributed to our understanding of early embryogenesis and the biology of cells forming the egg cylinder. These contributions could be summarized as follows:

a. Embryonic ectoderm is the only part of the egg cylinder that can give rise to EC cells (Diwan and Stevens, 1976).

b. Embryonic ectodermal cells can give rise to EC cells only before the onset on neurulation (Damjanov *et al.*, 1971b). EC can be produced only from 6- and 7-day-old egg cylinders. The older embryos do not contain undifferentiated developmentally pluripotent cells because all the ectodermal cells have become developmentally committed and will invariably differentiate into somatic tissues upon transplantation to extrauterine sites.

c. The inbred mouse strains differ with regard to their ability to form EC cells upon ectopic grafting (Solter *et al.*, 1979). The reasons for these strain differences have not been elucidated, although we have shown that even the teratocarcinoma «non-permissive» strain embryos may give rise to EC cells upon transplantation to F_1 hybrids (Solter *et al.*, 1981). This indicates that the developmental fate of the transplanted ectodermal cells in the egg cylinder is determined in part genetically and in part epigenetically by factors operating in the adult graft recipient animal. Hypothetically these epigenetic factors could have an active stimulatory growth-promoting effect on the undifferentiated cells; they could inhibit differentiation, or they could prevent the destruction of undifferentiated embryonic cells in the graft. The actual growth/differentiation inhibiting/promoting influences that the adult organism exerts on the grafted embryo have not been identified.

d. Rat ectodermal cells cannot form EC cells upon transplantation underneath the kidney capsule of syngeneic adult hosts (Skreb *et al.*, 1976). At the egg cylinder stage of development the rat ectodermal cells have apparently become developmentally committed and cannot proliferate and retain the undifferentiated embryonic phenotype in the heterotopic grafts. Rat egg cylinders explanted *in vitro* reflect this limited growth potential of rat embryonic cells (Skreb *et al.*, 1986).

e. Rat egg cylinders may give rise to yolk sac carcinomas (Damjanov *et al.*, 1977). Although yolk sac carcinomas may be occasionally derived from mouse egg cylinders as well (Damjanov and Solter, 1973) most of these tumors appear after a very long interval following the transplantation of the embryo (van Berlo *et al.*, 1990). On the other hand the rat yolk sac tumors develop much faster and can be produced in 2-3 months following embryonic transplantation. The reasons for the propensity of rat egg cylinders to form yolk sac tumors are not known. Since there are no reports on the *in vitro* cloning of neoplastic yolk sac cells directly from the egg cylinder, the grafting of embryos is still the method of choice for producing yolk sac tumors from rat egg cylinders.

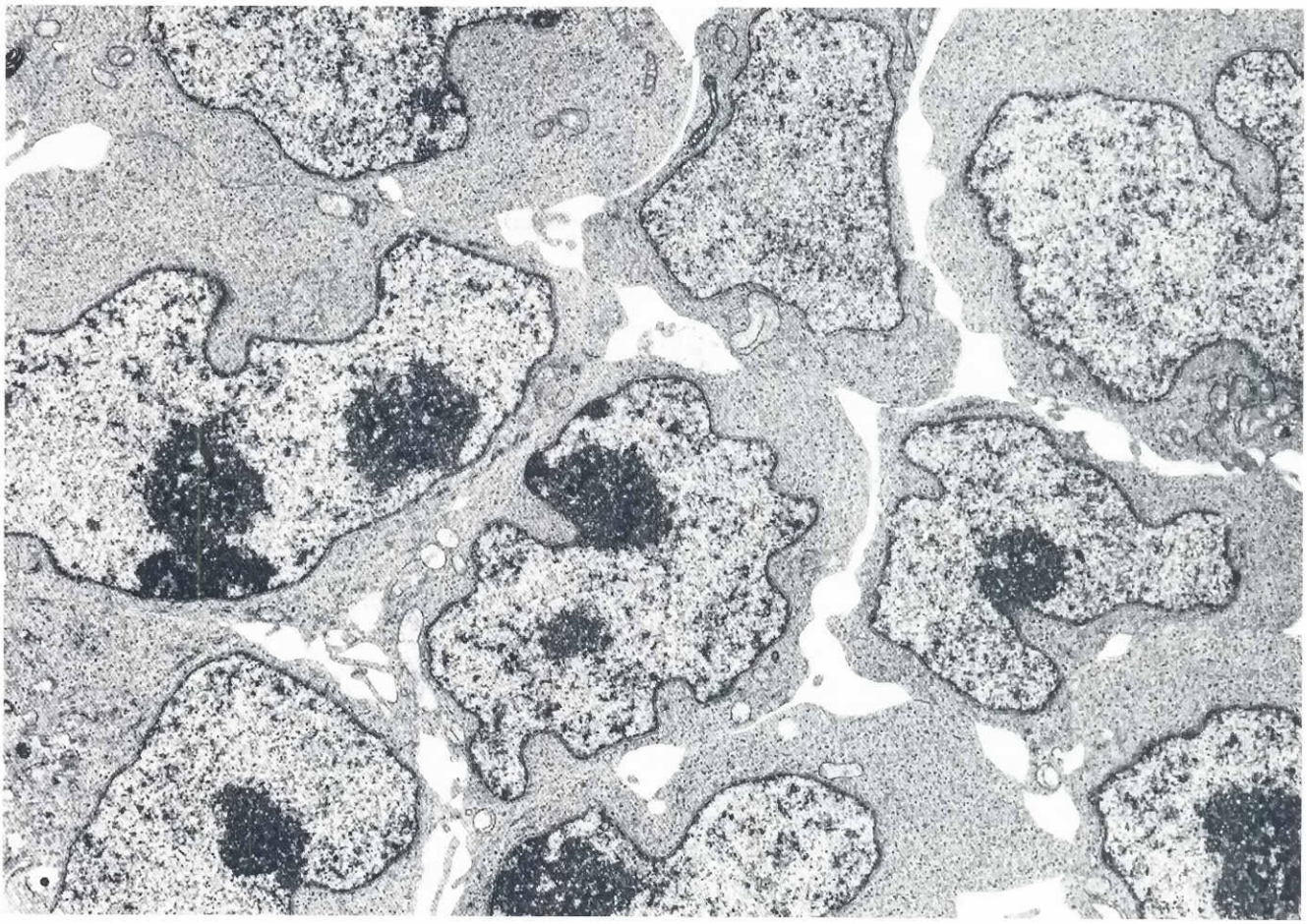


Fig 14. Electron microscopy of the mesoderm of a 7.5-day mouse egg cylinder. *The cells are loosely arranged. In the cytoplasm of most cells, there are only a few organelles besides the free ribosomes. x6000.*

Mouse teratocarcinomas derived from transplanted embryos or germ cells have been used extensively as a source of embryonic cells (lists of cells may be found in Silver *et al.*, 1983). Teratocarcinoma-derived cells have been compared with embryonic cells from early stages of development and a considerable similarity has been found between the normal embryonic cells and cells cloned *in vitro*. Developmentally pluripotent cells corresponding to the inner cell in the ICM or the ectoderm of the mouse egg cylinder have received most attention (Martin, 1980). However, cell lines corresponding to parietal yolk sac (Lehman *et al.*, 1974) or trophectoderm (Damjanov *et al.*, 1985) have also been isolated and used as experimental models for studying the function of equivalent embryonic cells.

Conclusion and Summary

The major restriction of the regulative capacity of embryonic cells that occurs at the time of mesoderm formation in the egg cylinder is one of the most significant events preceding organogenesis. The loss of developmental pluripotentiality, which is a marker of early embryonic cells, is accompanied by the developmental determination of evolving clones of committed embryonic cells. During these stages of early postimplantation development the cells pass the point of no return, and enter a highly regimented phase of morphogenesis.

The egg cylinder has been extensively characterized by morphological means. Despite numerous studies and several approaches designed to elucidate the key issues, many critical questions remain unanswered. The layers forming the egg cylinder, and conventionally called ectoderm, mesoderm and endoderm, have not yet been fully characterized. It is nevertheless clear that they do not represent the final germ layers postulated by classical embryologists, and are thus more or less a morphological concept (Svajger *et al.*, 1986). Each of the germ layers of the egg cylinder consists of a heterogeneous population of cells which undergo extensive mixing and interact with neighboring cells of the same germ layer as well as with those from adjacent layers. The interaction is mediated by cell-to-cell contact and soluble morphogens. The nature of these inducers of differentiation or promoters of growth remains poorly understood. The species differences represent an important confounding element barring any major generalizations or extrapolations of data from one species to another.

Morphological, biochemical and immunochemical characterization of immortalized or neoplastic embryonic cells derived from the egg cylinder has contributed to the better understanding of the function of equivalent cells in the developing embryo. Manipula-

tions of embryos *in vitro* and their transplantation *in vivo* or explantation *in vitro* have provided important insights into the developmental potential of embryonic cells forming anatomically distinct regions of the egg cylinder. Various spatial and chronological as well as biochemical determinants of development have been explored in these experiments, but the complexity of cell-to-cell interactions, the intricacy of various morphogenetic events, and the fluidity of emerging developmental fields leaves many questions open (Svajger *et al.*, 1981).

In summary, our research has been revolving in a circle: from characterization of embryos *in vivo*, we have continued studying embryos *in vitro*, or in xenografts *in vivo*. These experiments led us to study teratocarcinomas and EC cell lines derived from these tumors. Teratocarcinoma taught us in turn new facts about the embryos. The egg cylinder was thus both the beginning and the finish line of our studies. It was a most valuable source of cells, the study of which, in turn, contributed to the better understanding of its own morphogenesis and the biology of its constituent embryonic cells.

KEY WORDS: *egg cylinder, ectoderm, mesoderm, endoderm, embryonal carcinoma, teratocarcinoma*

Aknowledgments

The original work reviewed here was supported by U.S.P.H.S. grants HD-21355 AA-07186, and the Self Managing Community of Interest of S.R. Croatia through the funds made available to the US-Yugoslav Joint Board on Scientific and Technological Cooperation (No. 702). The secretarial help was provided by Sandra Dixon-Ross. Figure 8 was provided by Dr. Bruce Fenderson and Figure 11 by Dr. Antonio Martínez-Hernández.

References

ADAMSON, E.D. (1986). Cell-lineage-specific gene expression in development. In *Experimental Approaches to Mammalian Embryonic Development* (Eds. J. Rossant and R.A. Pedersen). Cambridge University Press, Cambridge, pp. 321-364.

AMENTA, P.S., CLARK, C.C. and MARTINEZ-HERNANDEZ, A. (1983). Deposition of fibronectin and laminin in the basement membrane of the rat parietal yolk sac: immunohistochemical and biosynthetic studies. *J. Cell Biol. 96:* 104-111.

BATTEN, B.E. and HAAR, J.L. (1979). Fine structural differentiation of germ layers in the mouse at the time of mesoderm formation. *Anat. Rec. 194:* 125-142.

BECK, F., LLOYD, J.B. and GRIFFITHS, A. (1967). A histochemical and biochemical study of some aspects of placental function in the rat using maternal injection of horseradish peroxidase. *J. Anat. 101:* 461-478.

BEDDINGTON, R.S.P. (1981). An autoradiographic analysis of the potency of embryonic ectoderm in the 8th day postimplantation mouse embryo. *J. Embryol. Exp. Morphol. 64:* 87-104.

BEDDINGTON, R.S.P. (1982). An autoradiographic analysis of tissue potency in different regions of the embryonic ectoderm during gastrulation in the mouse. *J. Embryol. Exp. Morphol. 69:* 265-285.

BEDDINGTON, R.S.P. (1983). Histogenetic and neoplastic potential of different regions of the mouse embryonic egg cylinder. *J. Embryol. Exp. Morphol. 75:* 189-204.

BEDDINGTON, R. (1986). Analysis of tissue fate and prospective potency in the egg cylinder. In *Experimental Approaches to Mammalian Embryonic Development* (Eds. J. Rossant, and R.A. Pedersen). Cambridge University Press, New York, pp. 121-147.

BRINSTER, R.L. (1974). The effects of cells transferred into mouse blastocyst on subsequent development. *J. Exp. Med. 140:* 1049-1056.

BULIC-JAKUS, F., SKREB, N., JURIC-LEKIC, G. and SVAJGER, A. (1990). Transferrin enhances lentoid differentiation in rat egg cylinders cultivated in a chemically defined medium. *Int. J. Dev. Biol. 34:* 275-279.

DAMJANOV A., WEWER, U.M. TUMA, B. and DAMJANOV, I. (1990). Basement membrane components secreted by mouse yolk sac carcinoma cell lines. *Differentiation 45:* 84-95.

DAMJANOV, I. (1990). Heterogeneity of basement membranes in normal and pathologically altered tissues. *Virchows Arch. A 416:* 185-188.

DAMJANOV, I., DAMJANOV, A. and ANDREWS, P.W. (1985). Trophectodermal carcinoma: mouse teratocarcinoma-derived tumor stem cell differentiating into trophoblastic and yolk sac elements. *J. Embryol. Exp. Morphol. 86:* 125-141.

DAMJANOV, I., DAMJANOV, A. and DAMSKY, C.H. (1986). Developmentally regulated expression of the cell-cell adhesion glycoprotein cell-CAM 120/80 in periimplantation mouse embryos and extraembryonic membranes. *Dev. Biol. 116:* 194-202.

DAMJANOV, I., SKREB, N. and SELL, S.C. (1977). Origin of embryo-derived yolk sac carcinoma. *Int. J. Cancer, 19:* 526-530.

DAMJANOV, I. and SOLTER, D. (1973). Yolk sac carcinoma grown from mouse egg-cylinder. *Arch. Pathol. 95:* 182-184.

DAMJANOV, I. and SOLTER, D. (1974). Experimental teratoma. *Curr. Top. Pathol. 59:* 69-129.

DAMJANOV, I., SOLTER, D. and SKREB, N. (1971a). Enzyme histochemistry of experimental embryo-derived teratocarcinomas. *Z. Krebsforsch. Klin. O. 76:* 249-256.

DAMJANOV, I., SOLTER, D. and SKREB, N. (1971b). Teratocarcinogenesis as related to the age of embryos grafted under the kidney capsule. *Roux Arch. Dev. Biol. 167:* 288-290.

DEFELICI, M. and DOLCI, S. (1987). Cellular interactions of mouse fetal germ cells in *in vitro* systems. *Curr. Top. Dev. Biol. 23:* 147-162.

DIWAN, S.B. and STEVENS, L.C. (1976). Development of teratomas from ectoderm of mouse egg cylinders. *J. Natl. Cancer Inst. 57:* 937-942.

DZIADEK, M. (1978). Modulation of alphafetoprotein synthesis in the early postimplantation mouse embryo. *J. Embryol. Exp. Morphol. 46:* 135-146.

EDDIE, E.M., CLARK, J.M., GONG, D. and FENDERSON, B.A. (1981). Origin and migration of primordial germ cells in mammals. *Gamete Res. 4:* 333-362.

ENDERS, A.C. and SCHLAFKE, S. (1967). A morphological analysis of the early implantation stages in the rat. *Am J. Anat. 120:* 185-226.

ELLINGTON, S.K.L. (1985). A morphological study of the development of the allantois of rat embryos *in vitro*. *J. Anat. 142:* 1-11, 1985.

EVANS, M.J. and KAUFMAN, M.H. (1981). Establishment in culture of pluripotent cells from mouse embryos. *Nature 192:* 154-156.

FENDERSON, B.A., EDDY, E.M.A and HAKOMORI, S.-I. (1988). The blood group I antigen defined by monoclonal antibody C6 is a marker of early mesoderm during murine embryogenesis. *Differentiation 38:* 124-133.

FENDERSON, B.A., EDDY, E.M. and HAKOMORI, S.-I. (1990). Glycoconjugate expression during embryogenesis and its biological significance. *Bioessays 12:* 173-179.

FENDERSON, B., HOLMES, E.H., FUKUSHI, Y. and HAKOMORI, S.-I. (1986). Coordinate expression of X and Y haptens during murine embryogenesis. *Dev. Biol. 114:* 12-21.

FOX, N., DAMJANOV, I., KNOWLES, B.B. and SOLTER, D. (1984). Stage specific embryonic antigen 3 as a marker of visceral embryonic endoderm. *Dev. Biol. 103:* 263-266.

FOX, N., DAMJANOV, I., MARTINEZ-HERNANDEZ, A., KNOWLES, B.B. and SOLTER, D. (1981). Immunohistochemical localization of the early embryonic antigen (SSEA-1) in postimplantation mouse embryos and fetal and adult tissues. *Dev. Biol. 83:* 391-398.

FRANKE, W.W., GRUND, C., KUHN, C., JACKSON, B.W. and ILLMENSEE, K. (1982). Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin filaments. *Differentiation. 23:* 43-59.

GARDNER, R.L. (1982). Investigation on cell lineage and differentiation in the extraembryonic endoderm of the mouse embryo. *J. Embryol. Exp. Morphol. 68:* 175-198.

GARDNER, R.L. (1985). Clonal analysis of early mammalian development. *Philos. Trans. R. Soc. Biol. 312:* 163-178.

GARDNER, R.L. (1986). Cell mingling during mammalian embryogenesis. *J. Cell Sci. (Suppl.) 4:* 337-356.

GARDNER, R.L. and ROSSANT, J. (1979). Investigation of the fate of 4.5 day post-coitum mouse inner cell mass cells by blastocyst injection. *J. Embryol. Exp. Morphol. 52:* 141-152.

HERTIG, A.T., ROCK, J. and ADAMS, E.C. (1956). A description of 34 human ova within the first 17 days of development. *Am. J. Anat. 98:* 435-498.

HSU, Y.-C. (1979). *In vitro* development of individually cultured whole mouse embryos from blastocyst to early somite stage. *Dev. Biol. 68:* 453-461.

HOGAN, F.L.M., BARLOW, D.P. and TILLY, R. (1983). F9 teratocarcinoma cells as a model for the differentiation of parietal and visceral endoderm in the mouse embryo. *Cancer Surv. 2:* 115-140.

HOGAN, B.M.L. and NEWMAN, R. (1984). A scanning electron microscope study of the extraembryonic endoderm of the 8th day mouse embryo. *Differentiation 26:* 138-143.

HOGAN, B. and TILLY, R. (1978a). *In vitro* development of inner cell masses isolated immunosurgically from mouse blastocysts. I. Inner cell masses from 3.5 day p.c. blastocysts incubated for 24h before immunosurgery. *J. Embryol. Exp. Morphol. 45:* 93-205.

HOGAN, B. and TILLY, R. (1978b). *In vitro* development of inner cell masses isolated immunosurgically from mouse blastocyst. II. Inner cell masses from 3.5- to 4.0-day p.c. blastocyst. *J. Embryol. Exp. Morphol. 45:* 107-121.

HOGAN, B.L.M. and TILLY, R. (1981). Cell interactions and endoderm differentiation in cultured mouse embryos. *J. Embryol. Exp. Morphol. 62:* 379-394.

HUBER, G.C. (1915). The development of the albino rat, Mus norvegicus albinus. I. From the pronuclear stage to the state of mesoderm anlage; end of the first to the end of the ninth day. *J. Morphol. 26:* 247-358.

JACOBSON, A.G. and SATER, A.K. (1988). Features of embryonic induction. *Development 104:* 341-359.

JENSEN, M., LLOYD, J.B., KOSZALKA, T.R., BECKMAN, D.A. and BRENT, R.L. (1989). Preparation and developmental toxicity of monoclonal antibodies against rat visceral yolk sac antigens. *Teratology 40:* 505-511.

KIRBY, D.R.S., POTTS, D.M. and WILSON, I.B. (1967). On the orientation of the implanting blastocyst. *J. Embryol. Exp. Morphol. 17:* 527-532.

LANE, E.B., HOGAN, B.L.M., KURKINEN, M. and GARRELS, J.I. (1983). Coexpression of vimentin and cytokeratins in parietal endoderm cells of the early mouse embryo. *Nature 303:* 701-704.

LAWSON, K.A., MENESES, J.J. and PEDERSEN, R.A. (1986). Cell fate and cell lineage in the endoderm of the presomite mouse embryo, studied with an intracellular tracer. *Dev. Biol. 115:* 325-339.

LAWSON, K.A. and PEDERSEN, R.A. (1987). Cell fate, morphogenetic movement and population kinetics of embryonic endoderm at the time of germ layer formation in the mouse. *Development 101:* 627-652.

LEHMAN, J.M., SPEERS, W.C., SWARTZENDRUBER, D.E. and PIERCE, G.B. (1974). Neoplastic differentiation. Characteristics of cell lines derived from a murine teratocarcinoma. *J. Cell Physiol. 84:* 13-20.

LEVAK-SVAJGER, B. and SVAJGER, A. (1971). Differentiation of endodermal tissues in homografts of primitive ectoderm from two-layered rat embryonic shields. *Experientia 27:* 683-684.

LEVAK-SVAJGER, B. and SVAJGER, A. (1974). Investigation of the origin of definitive endoderm in the rat embryo. *J. Embryol. Exp. Morphol. 32:* 445-459.

MARO, B., KUBIAK, J., GUETH, C., DE PENNART, H., HOULISTON, E., WEBER, M., ANTONY, C. and AGHION, J. (1990). Cytoskeleton organization during oogenesis, fertilization and preimplantation development of the mouse. *Int. J. Dev. Biol. 34:* 127-137.

MARTIN, G.R. (1980). Teratocarcinomas and mammalian embryogenesis. *Science 209:* 768-772.

MARTIN, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. USA 78:* 7634-7638.

MARTINEZ-HERNANDEZ, A. and AMENTA, P.S. (1983). The basement membrane in pathology. *Lab. Invest. 48:* 656-677.

MASON, I.J., TAYLOR, A., WILLIAMS, J.G., SAGE, H. and HOGAN, B.L. (1986). Evidence from molecular cloning that SPARC, a major product of mouse embryo parietal endoderm, is related to an endothelial cell "culture shock" glycoprotein of Mr 43000. *EMBO J. 5:* 1465-1472.

MINTZ, B. and FLEISCHMAN, R.A. (1981). Teratocarcinomas and other neoplasms as developmental defects in gene expression. *Adv. Cancer Res. 34:* 211-278.

MURAMATSU, T. (1988). Developmentally regulated expression of cell surface carbohydrates during mouse embryogenesis. *J. Cell. Biochem. 36:* 1-14.

NADIJCKA, M. and HILLMAN, N. (1974). Ultrastructural studies of the mouse blastocyst substages. *J. Embryol. Exp. Morphol. 32:* 675-695.

NAKATSUJI, N., SNOW, M.H.L. and WYLIE, C.C. (1986). Cinemicrographic study of the cell movement in the primitive-streak mouse embryo. *J. Embryol. Exp. Morphol. 96:* 99-109.

PARR, E.L., TUNG, H.N. and PARR, M.B. (1987). Apoptosis as the mode of uterine epithelial cell death during embryo implantation in mice and rats. *Biol. Reprod. 36:* 211-225.

PEDERSEN, R.A. (1986). Potency, lineage, and allocation in preimplantation mouse embryos. In *Experimental Approaches to Mammalian Embryonic Development* (Eds. J. Rossant and R.A. Pedersen). Cambridge University Press, Cambridge, New York, 1986, pp. 3-33.

PEDERSEN, R.A., SPINDLE, A.I. and WILEY, L.M. (1977). Regeneration of endoderm by ectoderm isolated from mouse blastocysts. *Nature 270:* 435-437.

PIENKOWSKI, M., SOLTER, D. and KOPROWSKI, H. (1974). Early mouse embryos: growth and differentiation *in vitro*. *Exp. Cell Res. 85:* 424-428.

PIERCE, G.B. JR., MIDGLEY, A.R., FELDMAN, J.D. and SRI RAM, J. (1962). Parietal yolk sac carcinoma: clue to the histogenesis of Reichert's membrane of the mouse embryo. *Am. J. Pathol. 41:* 549-566.

RODE, B., DAMJANOV, I. and SKREB, N. (1968). Distribution of acid and alkaline phosphatase activity in early stages of rat embryos. *Bull. Sci. Acad. Iugosl. 13:* 304.

ROSSANT, J. (1986). Development of extraembryonic cell lineages in the mouse embryo. In *Experimental Approaches to Mammalian Embryonic Development* (Eds. J. Rossant and R.A. Pedersen). Cambridge University Press. New York, pp. 97-120.

ROSSANT, J. (1987). Cell lineage analysis in mammalian embryogenesis. *Curr. Top. Dev. Biol. 23:* 115-146.

ROSSANT, J. and OFER, L. (1977). Properties of extraembryonic ectoderm isolated from postimplantation mouse embryos. *J. Embryol. Exp. Morphol. 39:* 183-194.

ROSSANT, J. and VIJH, K.M. (1980). Ability of outside cells from preimplantation mouse embryos to form inner cell mass derivatives. *Dev. Biol. 76:* 475-482.

SATO, M., OZAWA, M., HAMADA, H., KASAI, M., TOKUNAGA, T. and MURAMATSU, T. (1985). Cell surface markers to monitor the process of visceral endoderm differentiation from embryonal carcinoma cells: identification of the stage sensitive to high concentration of retinoic acid. *J. Embryol. Exp. Morphol. 88:* 165-182.

SCHLAFKE, S. and ENDERS, A.C. (1975). Cellular basis of interaction between trophoblast and uterus at implantation. *Biol. Reprod. 12:* 41-65.

SILVER, L.M., MARTIN, G.R. and STRICKLANDS, S. (Eds) (1983). *Teratocarcinoma Stem Cells*. Cold Spring Harbor Laboratory, Cold Spring Harbor.

SKREB, N. and BULIC., F. (1987). Partial differentiation of rat egg cylinders in serum-free and protein-free medium. *Dev. Biol. 120:* 584-586.

SKREB, N. and CRNEK, V. (1980). Development of embryo-derived teratoma *in vitro*. In *Differentiation and Neoplasia* (Eds. R.G. McKinnell, M.A. Di Bernardino, M. Blumenfeld and R.D. Bergad). Springer Verlag, Berlin, pp. 283-289.

SKREB, N., CRNEK, V. and DURST-ZIVKOVIC, B. (1983). Effects of various sera on cultured rodent embryonic shields. *Cell Differ. 12:* 27-32.

SKREB, N. and SVAJGER, A. (1973). Histogenetic capacity of rat and mouse embryonic shields cultivated *in vitro*. *Roux Arch. Dev. Biol. 173:* 228-234.

SKREB, N., SVAJGER, A. and LEVAK-SVAJGER, B. (1976). Developmental potentialities of the germ layers. In *Embryogenesis in Mammals* (Eds. K. Elliot and M. O'Connor). CIBA Foundation Symposium 40 (new series). Excerpta Medica, Elsevier, Amsterdam, pp. 27-39.

SMITH, J.C. (1989). Mesoderm induction and mesoderm inducing factors in early amphibian development. *Development 105:* 665-677.

SMITH, A.G., HEATH, J.K., DONALDSON, D.D., WONG, G.G., MOREAU, J., STAHL, M. and ROGERS, D. (1988). Inhibition of pluripotent embryonic stem cell differentiation by purified polypeptides. *Nature 336:* 688-690.

SOBIS, H., VAN HOVE, L. and VANDERPUTTE, M. (1982). Cellular events during early formation of yolk sac derived teratomas. *J. Embryol. Exp. Morphol. 70:* 225-240.

SOBOTTA, J. (1911). Die Entwicklung des Eies der Maus vom ersten Auftreten des Mesoderms an bis zur Ausbildung der Embryonalanlage und Auftreten der Allantois. *Arch. Mikr. Anat. 78:* 271-352.

SOLTER, D. and DAMJANOV, I. (1973). Explantation of embryonic parts of 7d mouse egg cylinders. *Experientia 29:* 701-703.

SOLTER, D. and DAMJANOV, I. (1979. Teratocarcinoma and the expression of oncodevelopmental genes. *Methods Cancer Res. 18:* 227-332.

SOLTER, D., DAMJANOV, I. and SKREB, N. (1970). Ultrastructure of mouse egg-cylinder. *Z. Anat. Entw.-gesch. 132:* 291-298.

SOLTER, D., DAMJANOV, I. and SKREB, N. (1972). Demonstrability of some oxidative enzymes in early rodent embryos with and without fixation. *Dev. Biol. 29:* 486-490.

SOLTER, D., DAMJANOV, I. and SKREB, N. (1973). Distribution of hydrolytic enzymes in early rat and mouse embryos - a reappraisal. *Z. Anat. Entw.-gesch. 139:* 119-126.

SOLTER, D., DOMINIS, M. and DAMJANOV, I. (1979). Embryo-derived teratocarcinoma. I. The role of strain and gender in the control of teratocarcinogenesis. *Int. J. Cancer 24:* 770-772.

SOLTER, D., DOMINIS, M. and DAMJANOV, I. (1981). Embryo-derived teratocarcinoma. III. Development of tumors from teratocarcinoma-permissive and non-permissive strain embryos transplanted to F_1 hybrids. *Int. J. Cancer 28:* 479-483.

SOLTER, D. and KNOWLES, B.B. (1975). Immunosurgery of mouse blastocyst. *Proc. Natl. Acad. Sci. USA 72:* 5099-5102.

SOLTER, D. and KNOWLES, B.B. (1978). Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci. USA 75:* 5565-5569.

SOLTER, D. and KNOWLES, B.B. (1986). Cell surface antigens of germ cells, embryos, and teratocarcinoma stem cells. In *Principles and Management of Testicular Cancer* (Ed. N. Javadpour). Thieme, New York, pp. 73-98.

SOLTER, D. and SKREB, N. (1968). La durée des phases du cycle mitotique dans différentes regions du cylindre-oeuf de la souris. *C.R. Acad. Séances (Paris) 267:* 659-661.

SOLTER, D., SKREB, N. and DAMJANOV, I. (1971). Cell cycle analysis in the mouse egg-cylinder. *Exp. Cell Res. 64:* 331-334.

STEVENS, L.C. (1967). The biology of teratomas. *Adv. Morphol. 6:* 1-31.

STEVENS, L.C. (1970). The development of transplantable teratocarcinomas from intratesticular grafts of pre- and post-implantation mouse embryos. *Dev. Biol. 21:* 364-382.

SVAJGER, A., LEVAK-SVAJGER, B., KOSTOVIC-KNEZEVIC, L. and BRADAMENTE, Z. (1981). Morphogenetic behavior of the rat embryonic ectoderm as a renal homograft. *J. Embryol. Exp. Morphol. 65 (Supp.):* 243-267.

SVAJGER, A., LEVAK-SVAJGER, B. and SKREB, N. (1986). Rat embryonic ectoderm as renal isograft. *J. Embryol. Exp. Morphol. 94:* 1-27.

TAM, P.P.L. (1989). Regionalization of the mouse embryonic ectoderm: allocation of prospective ectodermal tissues during gastrulation. *Development 107:* 55-67.

TAM, P.P.L. and BEDDINGTON, R.S.P. (1987). The formation of mesoderm tissues in the mouse embryo during gastrulation and early organogenesis. *Development 99:* 109-126.

TARKOWSKI, A.K. (1959). Experiments on the development of isolated blastomeres of mouse eggs. *Nature 184:* 1286-1287.

VANBERLO, R.J., OOSTERHUIS, J.W., SCHRIJNEMAKERS, E., SCHOOTS, C.J.F., DEJONG, B. and DAMJANOV, I. (1990). Yolk-sac carcinoma develops spontaneously as a late occurrence in slow-growing teratoid tumors produced from transplanted 7-day mouse embryos. *Int. J. Cancer. 45:* 153-155.

WAN, Y.-J., WU, T.-C., CHUNG, A.E. and DAMJANOV, I. (1984). Monoclonal antibodies to laminin reveal the heterogeneity of basement membranes in the developing and adult mouse tissues. *J. Cell Biol. 98:* 971-979.

WARTIOVAARA, J., LEIVO, I. and VAHERI, A. (1979). Expression of the cell surface-associated glycoprotein, fibronectin, in the early mouse embryo. *Dev. Biol. 69:* 247-257.

WEWER, U.M., TICHY, D., DAMJANOV, A., PAULSSON, M. and DAMJANOV, I. (1987). Distinct antigenic characteristics of murine parietal yolk sac laminin. *Dev. Biol. 121:* 397-407.

WU, T.-C., WAN, Y.-J. and DAMJANOV, I. (1981). Positioning of inner cell mass determines the development of mouse blastocysts *in vitro*. *J. Embryol. Exp. Morphol. 65:* 105-117.

WU, T.-C., WAN, Y.-J. and DAMJANOV, I. (1983). Fluorescein-conjugated *Bandeiraea simplicifolia* lectin as a marker of endodermal, yolk sac, and trophoblastic differentiation in the mouse embryo. *Differentiation 24:* 55-59.