

# Cell interactions and endoderm differentiation in cultured mouse embryos

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## SUMMARY

Morphological and biochemical evidence is presented that the visceral extraembryonic endoderm of the 6.5-day mouse embryo will differentiate into parietal endoderm when cultured in contact with extraembryonic ectoderm undergoing transition into trophoblast giant cells. Egg cylinders from 6.5-day embryos were dissected into embryonic and extraembryonic halves and cultured in suspension *in vitro* for up to 7 days. After 4 days, the endoderm cells of the extraembryonic fragments morphologically resemble parietal endoderm, are associated with a thick basement membrane and synthesize large amounts of the matrix proteins laminin and Type IV procollagen. A similar transition in phenotype is not seen in the endoderm of embryonic fragments, nor in visceral extraembryonic endoderm cells cultured in isolation. In another series of experiments, complete egg cylinders were dissected free of visceral endoderm overlying the extraembryonic ectoderm and then cultured *in vitro*. The visceral endoderm cells which recolonize the surface of the extraembryonic ectoderm develop a parietal endoderm phenotype and lay down a thick basement membrane. These results suggest that the differentiation of the extraembryonic endoderm of the early mouse embryo into visceral and parietal phenotypes can be influenced by local cell–cell or cell–substrate interactions, and is not determined solely by cell lineage.

## INTRODUCTION

The issue which we explore in this paper is the extent to which changes in local environment, brought about by *in vitro* culture, can influence the phenotype of extraembryonic endoderm cells of the early post-implantation mouse embryo. By the seventh day of development several morphologically and biochemically distinct endoderm populations are present in the mouse embryo (Fig. 1). The parietal endoderm (PE) is scattered over the inner surface of the trophoctoderm and lays down a thick basement membrane (Reichert's membrane), while the visceral endoderm forms a continuous epithelial layer over the egg cylinder. The visceral endoderm is further subdivided into the visceral extraembryonic endoderm (VEX End), overlying the extraembryonic ectoderm

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(Ex Ect), and the visceral embryonic endoderm (VE End), overlying the embryonic ectoderm (E Ect). Although no definite proof is yet available, it is generally believed that these extraembryonic endoderm cells are derived from a layer of primitive endoderm which delaminates from the inner cell mass at around 4.5 days *post coitum* (Enders, Given & Schlafke, 1978), and that until the formation of the definitive endoderm on the eighth day there is no further extensive recruitment of endoderm from embryonic ectoderm (for review, see Gardner, 1978). Assuming that the extraembryonic endoderm is all derived from the primitive endoderm, two models can be proposed for the differentiation of the parietal and visceral endoderm subpopulations. According to the first (Gardner & Papaioannou, 1975), all primitive endoderm cells have the capacity to express either the parietal or the visceral phenotype, but the choice depends on their position within the embryo; endoderm cells migrating on to the trophoctoderm differentiate in response to local signals into parietal endoderm, while those remaining in contact with the egg cylinder differentiate into visceral endoderm. According to the second model the fate of primitive endoderm cells is dependent on their lineage (Dziadek, 1979). The first cells to delaminate from the inner cell mass are programmed to be parietal endoderm. Before migrating on to the trophoctoderm these induce the inner cell mass to produce a second, separate, wave of primitive endoderm cells, this time committed to be visceral endoderm. This model does not allow the interconversion of cells with visceral and parietal phenotypes, nor the regeneration of parietal endoderm cells once the committed precursors have died out or been removed.

In this paper, we use several criteria to distinguish parietal from visceral endoderm. Morphologically, parietal endoderm cells of the normal embryo have few intercellular junctional complexes or microvilli, but do have an extensive endoplasmic reticulum filled with amorphous material. Visceral endoderm cells, on the other hand, form an epithelial layer and have apical intercellular junctional complexes and numerous microvilli, pinocytotic vacuoles and lysosomes (Solter, Damjanov & Skreb, 1970; Enders *et al.* 1978). Several biochemical differences have been reported between the two cell types (for review see Graham, 1979). However, in this paper we have scored only for the synthesis and release of high molecular weight, extracellular matrix proteins associated with the deposition of basement membrane material. Normal parietal endoderm cells are responsible for laying down Reichert's membrane (Minor *et al.* 1976; Hogan, Cooper & Kurkinen, 1980) and they synthesize large amounts of Type IV procollagen and laminin (Timpl *et al.* 1979) but no fibronectin (Adamson & Ayers, 1979; Hogan, 1980; Howe & Solter, 1980; Hogan *et al.* 1980). Normal visceral endoderm cells, on the other hand, are associated with only a thin basement membrane (Solter *et al.* 1970; Enders *et al.* 1978) and cells isolated from the 13.5-day visceral yolk sac synthesize very little Type IV procollagen and laminin but do make fibronectin (Adamson & Ayers, 1979; Hogan, 1980).

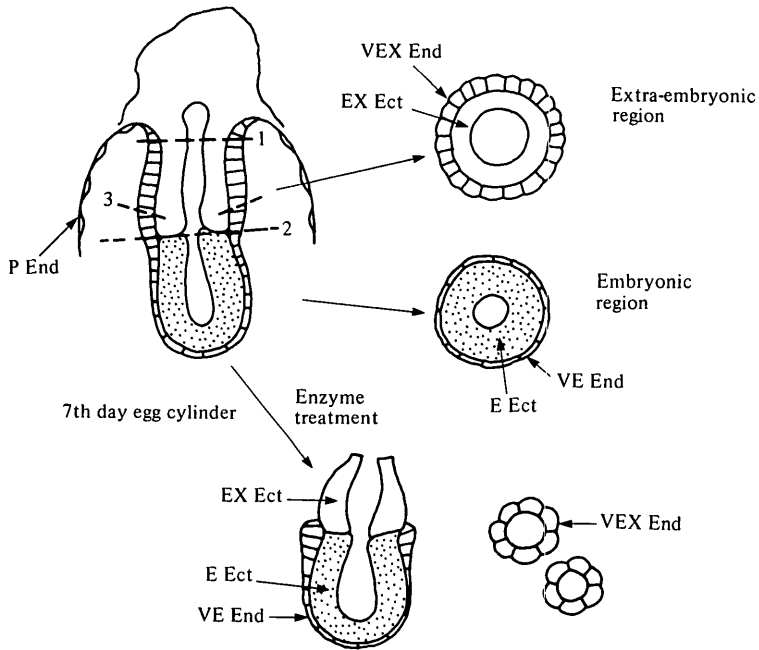


Fig. 1. Experimental procedure for the isolation and culture of egg cylinders from 7th day mouse embryos. Cuts in positions 1 and 2 were used for the isolation of extraembryonic and embryonic regions. Cuts in positions 1 and 3 were used to isolate egg cylinders without any visceral extraembryonic endoderm.

#### MATERIALS AND METHODS

##### *Embryo dissections*

Embryos of the C3H/He strain were isolated between 14.30 and 16.30 h on the 7th day of pregnancy, the day of the vaginal plug being the first day of pregnancy. Groups of approximately 20 embryos were dissected into warmed Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (Gibco Bio-cult) and 20 mM HEPES buffer, pH 7.4. Egg cylinders were then separated from the parietal endoderm and ectoplacental cone using tungsten needles to cut well into the extraembryonic portion. The egg cylinders were transferred to DMEM containing 10% foetal bovine serum and cut transversely to produce complete embryonic and extraembryonic fragments (see Fig. 1). For the removal of VEX End cells, the egg cylinders were washed once in DMEM without serum and incubated at 4 °C for 3 min in an enzyme solution containing 2.5% (w/v) pancreatin (BDH), 0.5% (w/v) trypsin (from bovine pancreas Type 111 Sigma), 0.5% (w/v) polyvinyl pyrrolidone (Sigma) in calcium-free DMEM (modified from Levak-Svajger, Svajger & Skreb, 1969). Before removing the VEX End with tungsten needles the egg cylinders were incubated for 1 h at 37 °C in DMEM with 10% foetal bovine serum to recover

from enzyme treatment. All embryo fragments were cultured in separate groups in 5 ml of DMEM containing 10% human cord serum in 50 mm bacteriological plastic petri dishes (Hogan & Tilly, 1978). Each experiment was repeated at least four times.

#### *Electron microscopy*

Embryonic fragments were fixed in 2.5% glutaraldehyde buffered with 0.06 M sodium cacodylate at pH 7.3 for 1 h, rinsed in three changes of cacodylate buffer and post-fixed in 1% osmium tetroxide buffered with veronal acetate at pH 7.3. Dehydration was carried out in a series of graded ethanols before embedding in Araldite medium. The sections for light microscopy were mounted on glass slides and stained with 1% toluidine blue in 1% borax solution. The sections for electron microscopy were stained with uranyl acetate solution followed by lead citrate and examined in a Siemens Elmiskop electron microscope.

#### *Radioactive labelling*

Embryo fragments and any detached clumps of cells were collected by sedimentation under gravity, washed twice in DMEM containing 1 µg/ml methionine and 10% dialysed foetal bovine serum and transferred to bacteriological plastic dishes in 1 ml of the same medium. After 1 h preincubation with 0.5 mM  $\alpha$ - $\alpha'$  dipyridyl (Sigma) to inhibit the hydroxylation and secretion of Type IV procollagen (Hogan *et al.* 1980) 100 µCi of [ $S^{35}$ ]methionine (specific activity 1000 Ci/mmol Radio-chemicals Centre, Amersham) was added and the incubation continued for a further 5–11 h at 37 °C.

#### *Isolation and analysis of high-molecular-weight proteins in the culture medium*

At the end of the incubation for radioactive labelling the medium was harvested and cells and embryo fragments removed by centrifugation. The supernatant was made 2 mM with phenylmethyl sulphonyl fluoride, 5 mM with n-ethyl maleimide and 4 mM with EDTA. Fifty µg/ml of carrier gelatin (Sigma, swine skin Type 1) was then added, followed by polyethylene glycol (Mol. wt. 6000, Koch-Light Laboratories Ltd) to a final concentration of 10% (w/v). After 2 h at 4 °C the precipitate was collected by centrifugation, washed twice with 70% aqueous ethanol, dissolved in electrophoresis sample buffer (Laemmli, 1970) with 5% w/v  $\beta$ -mercaptoethanol and boiled for 3 min. SDS polyacrylamide gel electrophoresis was carried out by the method of Laemmli (1970) using 5–10% gradient slab gels as described (Hogan, 1980).  $C^{14}$ -labelled protein standards (Radiochemicals Centre, Amersham) consisting of myosin (212 K daltons), phosphorylase-B (100 and 92.5 K daltons), bovine serum albumin (69 K daltons), ovalbumin (46 K daltons) and carbonic anhydrase (30 K daltons) were included in the gels. After fixation, the gels were impreg-

nated with EN<sup>8</sup>HANCE (New England Nuclear), dried and exposed to X-ray film (Kodak SB-5) at  $-70^{\circ}\text{C}$ .

## RESULTS

In the first series of experiments, 6.5-day egg cylinders were separated into extraembryonic and embryonic portions, as shown in Fig. 1. The cut surfaces rapidly closed over, and the fragments remained in healthy conditions over 7 days of suspension culture.

### *Extraembryonic visceral endoderm and ectoderm*

At the beginning of the culture period the visceral extraembryonic endoderm cells have a uniform morphology similar to that described for 6-day embryos by Solter *et al.* (1970). As shown in Figs. 2A, 3A the cells are joined apically by junctional complexes to form a columnar epithelial layer over the extraembryonic ectoderm, from which they are separated by only thin deposits of extracellular material (Fig. 3C). The endoderm cells have numerous apical microvilli, pinocytotic vacuoles and lysosomes, but contain little rough endoplasmic reticulum. After 4 days of culture a striking change occurs in the endoderm cells, which proliferate to form numerous grape-like clusters which detach from all over the embryo fragments and accumulate in the dish (Fig. 2B). Electron microscopy reveals that all the outer endoderm cells now have the morphology of parietal endoderm (Fig. 2C), with an extensive endoplasmic reticulum engorged with amorphous material. The cells have few microvilli or intercellular junctional complexes and are not organised into an epithelial layer. Most strikingly, they are separated from the extraembryonic ectoderm by a thick basement membrane similar in morphology to Reichert's membrane of the normal mouse embryo.

As demonstrated previously (Hogan, 1980; Hogan *et al.* 1980), and confirmed in Fig. 4, track 6, normal parietal endoderm cells isolated from 13.5-day embryos and cultured *in vitro* synthesize and release into the medium large amounts of the extracellular matrix protein, laminin. On electrophoresis under reducing conditions laminin migrates as three polypeptides of molecular weights 450 K (A), and 243 K and 233 K (B<sub>1</sub> and B<sub>2</sub> usually migrating as a closely spaced doublet). A fourth polypeptide chain of molecular weight 150 K (C), which is immunoprecipitated from the culture medium by antilaminin antibody, is also synthesized by normal parietal endoderm cells (Hogan *et al.* 1980). In order to separate clearly the B chains of laminin from the  $\alpha_1$  and  $\alpha_2$  chains of Type IV procollagen,  $\alpha$ - $\alpha^1$ -dipyridyl was added to the culture medium. Under these conditions synthesis of Type IV procollagen is inhibited and those underhydroxylated chains which are produced migrate faster on electrophoresis. Although synthesis of Type IV procollagen is reduced, it can be seen that parietal endoderm cells still produce much more of this matrix protein than visceral endoderm isolated from the 13.5-day visceral yolk sac, which also synthesizes very

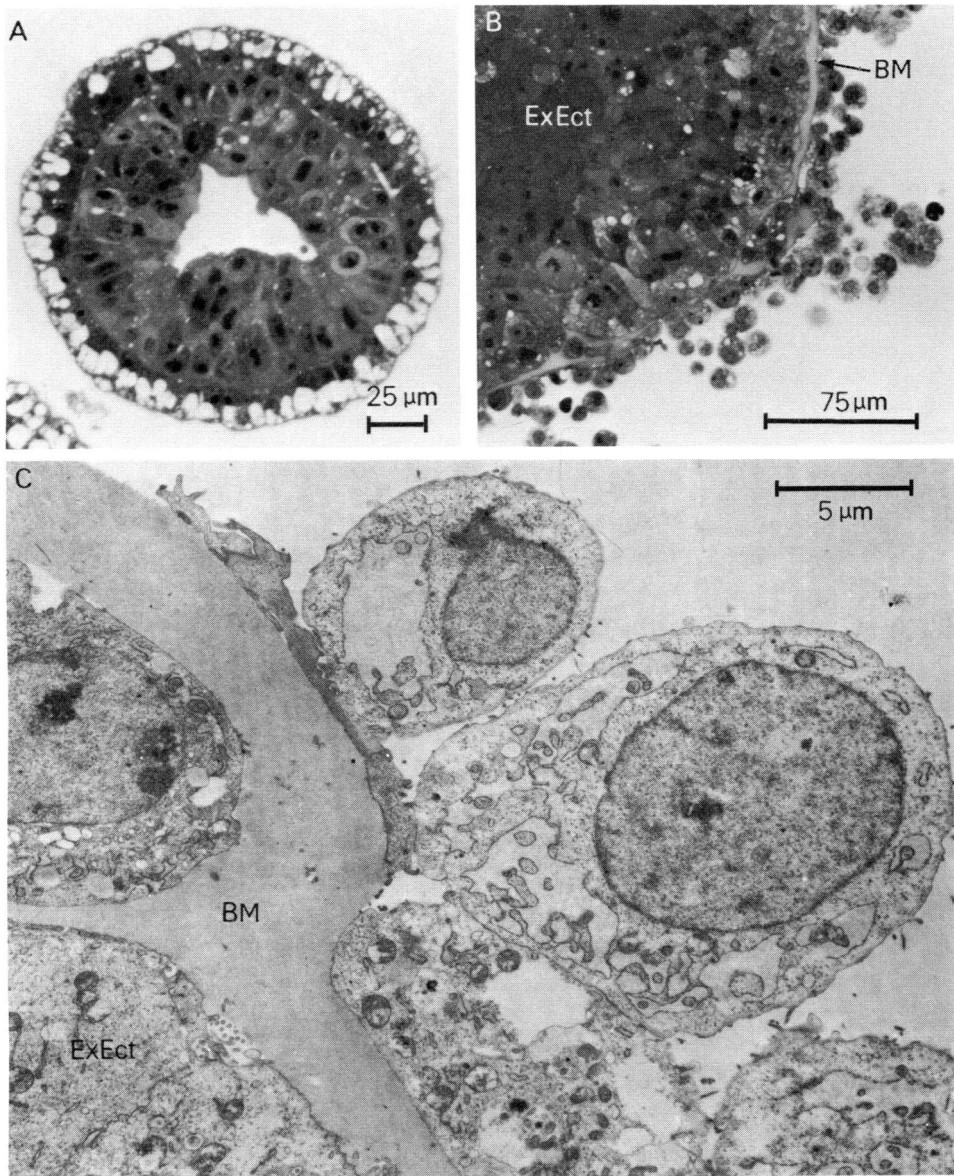


Fig. 2. Extraembryonic ectoderm and endoderm before and after culture for 4 days. (A) Section through the extraembryonic region shortly after isolation, showing the epithelial layer of VEX End cells surrounding the extraembryonic ectoderm (EX Ect). (B) Part of the extraembryonic region after 4 days in culture. Clusters of rounded cells are detaching from the endoderm layer which is now separated from the EX Ect cells by a thick basement membrane (BM). (C) Electron micrograph detail of endoderm cells in B. The outer cells have a parietal endoderm-like morphology with few microvilli and intercellular contacts, and a well-developed endoplasmic reticulum filled with amorphous material. A thick basement membrane (BM) lies between the endoderm cells and the EX Ect.

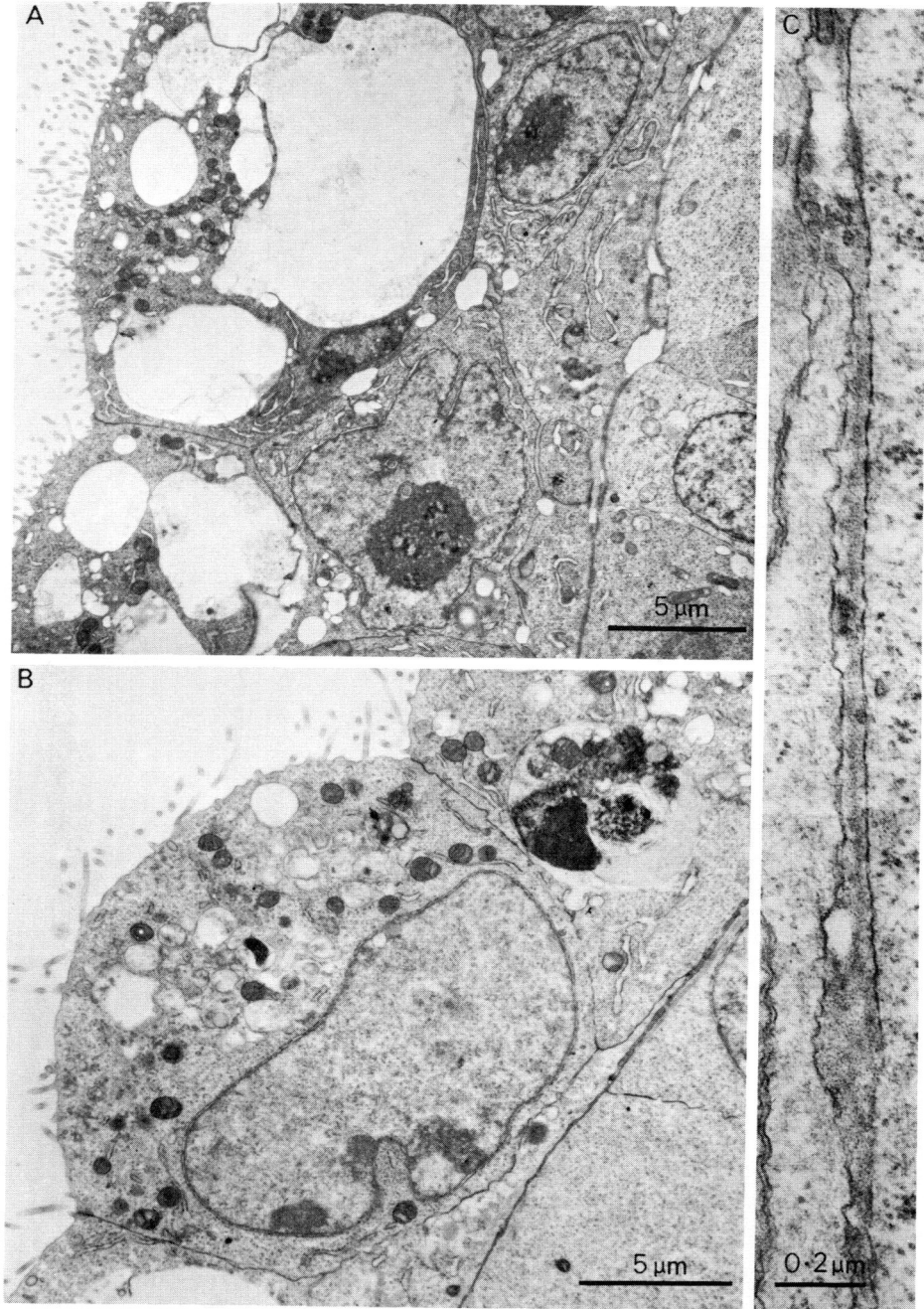


FIGURE 3

Visceral extraembryonic and embryonic endoderm cells of the 6.5-day embryo, before culture *in vitro*.

(A) Oblique section showing visceral extraembryonic endoderm cells overlying the extraembryonic ectoderm. Note the numerous microvilli, apical tight junctions and pinocytotic vacuoles. Scale bar 5  $\mu\text{m}$ .

(B) Visceral embryonic endoderm cells overlying the embryonic ectoderm. Scale bar 5  $\mu\text{m}$ .

(C) Detail of the thin basement membrane separating the visceral extraembryonic endoderm (left) and ectoderm (right). Scale bar 0.2  $\mu\text{m}$ .

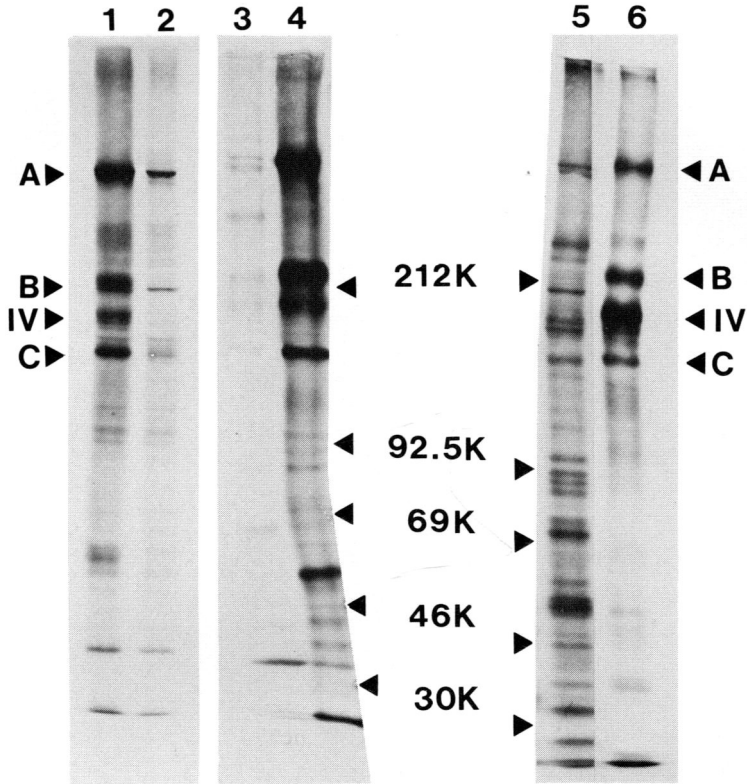


FIGURE 4

High-molecular-weight extracellular proteins synthesized by parietal and visceral endoderm of the 13.5-day embryo and by cultured embryo fragments. Cells and embryo fragments were preincubated with  $\alpha$ - $\alpha'$ -dipyridyl for 1 h and then labelled with [ $S^{35}$ ]methionine for the times shown. High molecular-weight proteins were precipitated from the medium with 10% (w/v) polyethylene glycol as described in Materials and Methods. Track 1, proteins synthesized by 12 egg cylinders denuded of VEX End and cultured for 7 days. The fragments were labelled for 5 h and one fifth of the total sample containing 2328 cpm applied to the gel, which was exposed to X-ray film for 28 days. Track 2, proteins synthesized by 12 egg cylinders denuded of VEX End and cultured for 4 days. Details as for track 1 except that the sample contained 1992 cpm. Track 3, proteins synthesized by 18 embryonic ectoderm and endoderm fragments after 4 days in culture. The fragments were labelled for 5 h and one half of the total sample, containing 2920 cpm, applied to the gel, which was exposed to X-ray film for 7 days. Track 4, proteins synthesized by 18 extraembryonic ectoderm and endoderm fragments after 4 days in culture. Details as for track 3 except that the sample contained 7390 cpm. Track 5, proteins synthesized by  $4 \times 10^5$  visceral endoderm cells isolated from 13.5-day embryos and labelled for 11 h. One fifth of the total sample, containing 17452 cpm was applied to the gel which was exposed to film for 48 h. Track 6, proteins synthesized by  $4 \times 10^5$  parietal endoderm cells. Details as for track 5 except that one twentieth of the total sample, containing 17500 cpm was applied to the gel which was exposed to film for 24 h. A, B and C indicate the position of polypeptides of molecular weights 450 K, 240 K and 150 K respectively, which can be immunoprecipitated with antilaminin antibody (see text). IV indicates the position of the under-hydroxylated  $\alpha_1$  and  $\alpha_2$  chains of Type IV procollagen which are still synthesized in the presence of the inhibitor  $\alpha$ - $\alpha'$ -dipyridyl (Hogan *et al.* 1980).



little of the A, B and C components of laminin (Fig. 4, track 5 and Hogan, 1980).

After four days in culture extraembryonic fragments synthesize and release into the medium large amounts of laminin (A, B and C) and Type IV procollagen, yielding a pattern of high-molecular-weight polypeptides almost identical to that of normal PE cells. A similar pattern was obtained after 7 days of culture (results not shown).

#### *Embryonic endoderm and ectoderm*

At the time of isolation the endoderm cells around the embryonic ectoderm appear as described for normal 6-day embryos by Solter *et al.* (1970) (Figs. 5A, 3B). The cells are more flattened than VEX End cells but have numerous microvilli, pinocytotic vacuoles and lysosomes, and only a sparse endoplasmic reticulum. Over the 7-day culture period about half of the embryonic fragments expand to form large vesicles containing blood islands and patches of beating muscle; the remainder do not expand into vesicles but invariably contain beating muscle. In neither case do large clusters of rounded cells ever bud off from the surface as described for the extraembryonic fragments. Light and electron microscopy shows that the cultured embryonic pieces contain large numbers of mesodermal cells (presumably derived from the embryonic ectoderm) underlying an organized epithelium of visceral endoderm-like cells. The endoderm cells are joined together by junctional complexes and only a thin basement membrane separates them from the mesoderm (Fig. 5B, C).

As shown in Fig. 4 track 3, after four days in culture very little laminin is synthesized and released into the medium by embryonic compared with extraembryonic fragments. A similar result was obtained after 7 days in culture (data not shown). It should be noted that tracks 3 and 4 of Figure 4 compare the same proportion of the total radioactively labelled high-molecular-weight proteins secreted into the culture medium by the same number of embryonic and extraembryonic fragments. However, a similar result was obtained if the same amount of radioactive protein from each sample was analysed on the same gel (data not shown).

The results of this first series of experiments suggested that visceral extraembryonic endoderm cells, and/or their descendants, are able to change their phenotype and differentiate into parietal endoderm when cultured in contact with extraembryonic ectoderm. However, we could not completely exclude the possibility that the extraembryonic fragments were contaminated near their cut ends with a few parietal endoderm cells, although this seemed unlikely since the clusters of parietal endoderm-like cells which appeared in culture developed on all surfaces of the fragments rather than at one pole. In order to eliminate this possibility a second series of experiments was carried out in which the VEX End layer was dissected away from egg cylinders after brief proteolytic treatment, the rest of the egg cylinder being left intact.

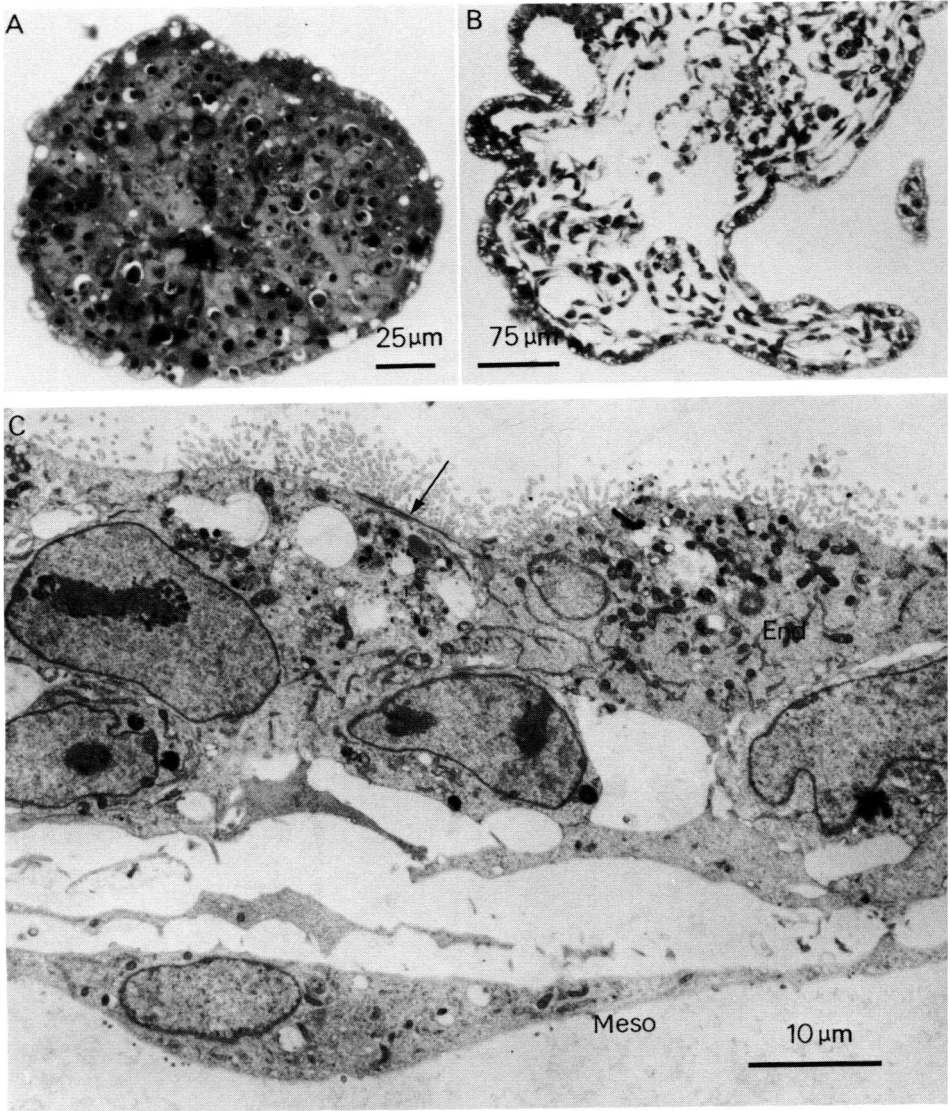


Fig. 5. Embryonic ectoderm and endoderm before and after culture for 4 days. (A) Section through the embryonic region shortly after isolation, showing the epithelial layer of VE End cells surrounding the embryonic ectoderm (E Ect). Scale bar 25  $\mu\text{m}$ . (B) Part of the embryonic region after 4 days of culture. The outer epithelial layer of endoderm cells is underlaid by a complex network of mesoderm cells. Scale bar 75  $\mu\text{m}$ . (C) Electron micrograph detail of endoderm cells in B. The outer endoderm cells (End) are joined by extensive junctional complexes (arrow), and have numerous microvilli and very little endoplasmic reticulum. There is very little basement membrane material between the endoderm cells and the underlying mesoderm (Meso). Scale bar 10  $\mu\text{m}$ .

*Egg cylinders denuded of VEX End*

After removal of the visceral extraembryonic endoderm the egg cylinders have the appearance shown in Fig. 6A. During the first 4 days of culture the embryonic portion either forms a large vesicle containing blood islands and beating muscle or remains small but with contractile areas. After 4 days the EX Ect cells become rounded, granular and giant, and at about the same time numerous grape-like clusters of small cells begin to appear near the junction of the embryonic and extraembryonic regions. Between 4 and 7 days of culture these cell clusters rapidly increase in number and bud off into the medium. Light and electron microscopy of the fragments after 7 days in culture shows a sharp transition in the morphology of the endoderm cells at the junction between the extraembryonic ectoderm and the mesoderm. The endoderm over the extraembryonic ectoderm resembles typical parietal endoderm and lies on top of a thick basement membrane. In contrast, the endoderm cells over the mesoderm are joined together by extensive intercellular junctional complexes and have the same morphology as endoderm cells in cultured embryonic fragments (Fig. 5C).

In a few cases the extraembryonic ectoderm detached from the embryonic region at the beginning of the culture period. These EX Ect fragments differentiated into giant cells after about four days of culture but did not develop clusters of endoderm cells.

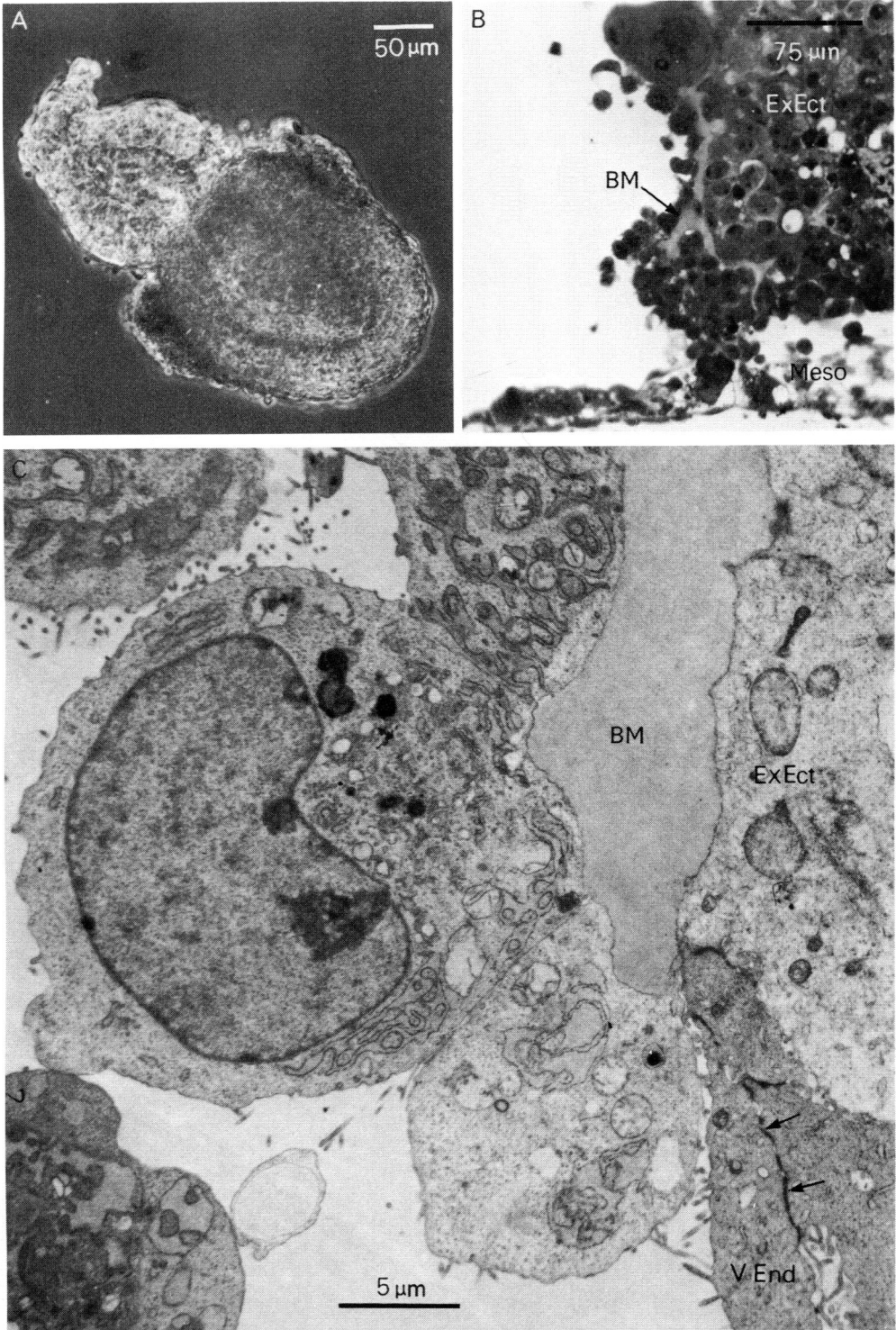
As shown in Fig. 4, egg cylinders dissected free of VEX End synthesize and release into the medium only small amounts of laminin and Type IV procollagen after 4 days in culture (track 2), but large amounts after 7 days when many more clusters of parietal endoderm-like cells were present (track 1).

*Visceral extraembryonic endoderm cultured alone*

The small fragments of endoderm cells isolated after enzyme treatment quickly round up into vesicles which slowly expand. When examined in the electron microscope after 7 days of culture these cells have a visceral endoderm morphology similar to that shown in Fig. 5C and are underlined by moderate amounts of basement membrane material.

DISCUSSION

The results of our first set of experiments suggest that in response to prolonged contact with extraembryonic ectoderm, which is undergoing conversion to trophoblast giant cells, visceral extraembryonic endoderm changes its phenotype and converts into cells with the characteristics of parietal endoderm. These characteristics are expressed both at the level of cell morphology, and biosynthetic activity (elaboration of a thick basement membrane and correlation with a high level of laminin synthesis). Similar evidence for the conversion of



visceral endoderm into parietal endoderm was obtained earlier by Solter & Damjanov (1973) and Solter *et al.* (1974) from experiments in which extra-embryonic fragments of 7-day egg cylinders were grafted under the kidney capsule of syngeneic mice. After four weeks all of the grafts contained parietal endoderm-like cells surrounded by large amounts of basement membrane material. More recently, Gardner has observed that visceral embryonic and extraembryonic endoderm cells isolated from post-implantation embryos can colonize the parietal yolk sac after injection into 3-5-day host blastocysts (R. L. Gardner, personal communication). The apparent transition of visceral into parietal endoderm observed under these various experimental conditions is inconsistent with any model for endoderm differentiation in which precursors committed to be either parietal or visceral endoderm differentiate consecutively from the inner cell mass (Dziadek, 1979). It is, on the other hand, compatible with models in which the phenotype of endoderm cells can be modulated by local cell-cell or cell-substrate interactions. However, in both our own initial experiments and those of Solter & Damjanov (1973) the possibility of contamination of the extraembryonic fragments with pre-existing PE cells could not be completely ruled out. Moreover, since the EX Ect cells develop under our culture conditions into a compact mass of trophoblast giant cells, it is possible to argue that the thick basement membrane which accumulates below the endoderm cells is not so much a characteristic product of parietal endoderm, analogous to Reichert's membrane in the normal embryo, as an artifact caused by a limitation on the surface available for attachment and spreading of endoderm cells making the same amount of matrix proteins as before.

In order to overcome these objections we carried out a second set of experiments in which most of the VEX End cells, and certainly all of the endoderm cells near the junction with Reichert's membrane, were dissected from the egg cylinders before culture. In these embryos, also, cells with the morphology of

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FIGURE 6

Egg cylinders dissected free of VEX End, before and after culture for 7 days.

(A) Phase-contrast micrograph of an egg cylinder shortly after removal of the VEX End cells. Scale bar 50  $\mu\text{m}$ .

(B) Section through an egg cylinder after 7 days in culture showing the junction between the extraembryonic ectoderm (EX Ect) and the mesoderm derived from the embryonic ectoderm (Meso). The endoderm cells over the EX Ect are rounded and loosely attached to the thick basement membrane (BM) between them and the EX Ect cells. The endoderm cells over the mesoderm at the bottom left of the picture form a continuous epithelium and do not lay down a thick basement membrane. Scale bar 75  $\mu\text{m}$ .

(C) Electron micrograph detail of a similar junctional region to that shown in B. The parietal endoderm-like cells over the EX Ect have a well developed endoplasmic reticulum and few intercellular junctional complexes. On the other hand, the visceral endoderm cells (V End) at the bottom right have numerous intercellular junctional complexes (arrows) and very little endoplasmic reticulum. Scale bar 5  $\mu\text{m}$ .

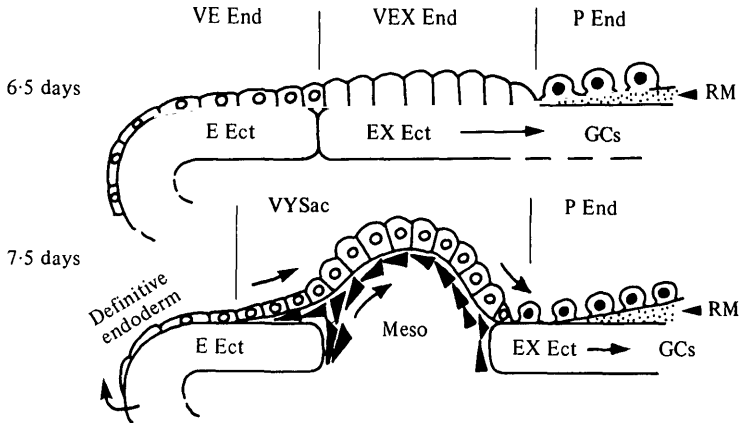


Fig. 7. Schematic representation of the changes in cell association and endoderm differentiation which may occur in the mouse embryo between 6.5 and 7.5 days *p.c.* Endoderm cells labelled with open circles react positively with specific anti-alphafoetoprotein (AFP) antiserum (Dziadek & Adamson, 1978), while parietal endoderm cells (P End) labelled with filled circles synthesize large amounts of laminin and Type IV procollagen, major components of the underlying Reichert's membrane (RM) (Hogan *et al.* 1980). At the time of gastrulation extraembryonic endoderm (EX Ect) withdraws from beneath the visceral extraembryonic endoderm (VEX End) and is replaced by mesoderm (Meso). As a result of the removal of the inhibiting influence of the EX Ect (Dziadek, 1978), and possibly stimulated by signals from the mesoderm, those VEX End cells which contribute to the visceral yolk sac (VY Sac) begin to synthesize AFP. Some VEX End cells may be recruited into the parietal endoderm population as a result of coming into contact with giant cells (GCs) derived from the withdrawing EX Ect. At the same time the visceral embryonic endoderm (VE End) around the embryonic ectoderm (E Ect) is replaced by the definitive endoderm delaminated from the ectoderm (Snell & Stevens, 1975).

parietal endoderm and associated with a thick basement membrane and a high rate of laminin synthesis appeared between 4 and 7 days in culture, but only in contact with the extraembryonic ectoderm. It is unclear whether the endoderm cells which recolonize the surface of the EX Ect and assume the parietal phenotype are derived from fully differentiated visceral endoderm cells or from immature stem cells intercalated in the endodermal epithelium. It is also possible that the signals promoting the conversion to parietal endoderm are generated by the trophoblast giant cells differentiating from the extraembryonic ectoderm, rather than by the EX Ect itself. However, what is clear is that at least some visceral extraembryonic endoderm cells can be converted into parietal endoderm in response to prolonged contact with extraembryonic ectoderm. This result complements the earlier studies of Dziadek (1978) on the modulating effect of extraembryonic ectoderm on the accumulation of alphafoetoprotein (AFP), by extraembryonic endoderm cells of the 7th and 8th day embryo. At this stage AFP is accumulated only by visceral embryonic endoderm and, later, by endoderm cells of the visceral yolk sac, but is not present in parietal endoderm at any stage (Dziadek & Adamson, 1978). Using intracellular immuno-

peroxidase staining as a specific probe, Dziadek (1978) found that both visceral embryonic and extraembryonic endoderm cells accumulated and presumably synthesized AFP when cultured in isolation, but did not do so when cultured in contact with extraembryonic ectoderm. From these and other observations Dziadek concluded that EX Ect inhibits the expression of AFP by visceral endoderm, as a result of signals transmitted through intercellular contact.

Taken together, our results and those of Dziadek (1978) show that the pattern of gene expression in the visceral extraembryonic endoderm of the 7th day embryo can be modulated in response to altered conditions. Although these alterations were brought about experimentally, the same group of cells in the normal embryo is exposed to dramatic changes in cell associations as a result of gastrulation on the 8th day of development, when the extraembryonic ectoderm withdraws towards the ectoplacental cone and is replaced by extraembryonic mesoderm generated at the primitive streak (Fig. 7). Thus, some VEX End cells which were previously inhibited from synthesizing alphafoetoprotein by extraembryonic endoderm may be induced to do so by contact with extraembryonic mesoderm if they are incorporated into the visceral yolk sac. At the same time, the endoderm cells may switch from Type IV procollagen to Type I collagen production (Adamson & Ayers, 1979). Likewise, some VEX End cells may be recruited into parietal endoderm, and switch to high levels of Type IV procollagen and laminin synthesis, as a result of contact with trophoblast giant cells in the chorion and ectoplacental cone. While it seems possible that local environmental factors do play an important role in endoderm differentiation in the developing embryo, it remains to be seen whether these effects are mediated through cell contacts, or through components of the extracellular matrix (see, for example, Meier & Hay, 1975; Lash & Vasan, 1978; Gospodarowicz, Delgado & Vlodavsky, 1980; Nathanson & Hay, 1980; Adamson, 1981).

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