

# Gap Junctional Communication in the Extraembryonic Tissues of the Gastrulating Mouse Embryo

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**Abstract.** We characterized gap junctional communication in the extraembryonic tissues of the 7.5-d gastrulating mouse embryo. At this stage of development, the extraembryonic tissues form a large part of the conceptus, and link the embryo proper to the maternal tissue. Using Lucifer yellow injections, cells in most extraembryonic tissues were observed to be very well dye coupled, the only exception being the peripheral regions of the ectoplacental cone. Of particular interest was the fact that no dye coupling was detected between the three major extraembryonic tissues. Thus, the extraembryonic ectoderm (EEC), the extraembryonic endoderm (EEN), and the ectoplacental cone (EPC) corresponded to separate communication compartments, with the EPC being further subdivided into three compartments. Interestingly, the EEN was ob-

served to exhibit a very low level of dye coupling with the adjacent visceral embryonic endoderm (EN), and consistent with the latter dye coupling results was the finding that the EEN was ionically coupled to the EN, but not with any other extraembryonic tissues. However, in the EPC, ionic coupling studies show that the central region was well coupled ionically to the EEC, but only weakly coupled to the peripheral EPC. These findings, in conjunction with our previous study (1988. *J. Cell Biol.* 107:241-255), demonstrate that the 7.5-d mouse conceptus is subdivided into at least nine major Lucifer yellow-delineated communication compartments, with ionic coupling across some of these compartments effectively unifying the embryo into two large domains corresponding to the embryo proper and the major extraembryonic tissues.

**G**AP junctions are membrane channels that provide an intercellular pathway for the efficient but passive exchange of ions, metabolites, and other molecules <1,000–1,500 daltons (13). They can be detected functionally by either monitoring for the intercellular passage of ions (ionic coupling), or by observing the cell-to-cell spread of membrane impermeant fluorescent tracers (dye coupling). Using these two techniques, gap junctional communication has been detected in many types of embryonic and adult cells from a large number of multicellular organisms (1, 3). It has been suggested that the ubiquitous presence of gap junctions from early stages of embryogenesis is consistent with a role in growth regulation, development, and/or pattern formation (13, 21). In particular, it has been suggested that among substances transferred through such channels, some could directly or indirectly specify positional information in a developing embryo (1, 2, 3, 10, 12, 21), and thus play a role in regulating pattern formation. The mammalian embryo, especially that of the mouse, constitutes a well-characterized developmental system that can be used to test the latter hypothesis.

Earlier studies by Lo and Gilula (11) have shown that in the early mouse embryo, gap junctional communication first becomes established at the 8-cell stage, coinciding with the time of compaction and just before the time of trophectoderm

determination (8). This pattern of complete coupling breaks down shortly after implantation, as observed with blastocysts implanted and cultured in vitro (12). This was seen as the selective loss of dye coupling, while ionic coupling persisted between the inner cell mass (ICM)<sup>1</sup> and the trophectoderm. As development proceeded, dye coupling in the ICM was observed to be further segregated into additional subcompartments (12). Consistent with and further extending these earlier findings are our recent results obtained with Lucifer yellow dye injections into the 7.5-d postimplantation embryo. These studies showed that with gastrulation, each of the newly formed germ layers of the embryo proper corresponded to a dye restricted communication compartment, with ionic coupling again persisting across the dye-delineated compartment boundaries (7). In addition, each germ layer was also observed to be further subdivided into smaller communication compartment domains.

In the present study, we characterized the pattern of gap junctional communication in extraembryonic regions of the 7.5-d mouse embryo. At this early postimplantation stage of development, the extraembryonic tissues constitute a large portion of the conceptus and begin to link the embryo proper

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1. *Abbreviations used in this paper:* EEC, extraembryonic ectoderm; EEN, extraembryonic endoderm; EEM, extraembryonic mesoderm; EN, embryonic endoderm; EPC, ectoplacental cone; EPC-C, central region of EPC; EPC-L, lateral peripheral region of EPC; EPC-U, upper peripheral region of EPC; ICM, inner cell mass; M, mesoderm.

to the maternal tissues. These cells are largely derived from the trophoderm lineage and also partly from the primitive ectoderm (e.g., the extraembryonic mesoderm). Eventually they give rise to the chorioallantoic placenta and the yolk sac membranes (17). That the extraembryonic constituents of the conceptus may play an important role in the development of the embryo proper is indicated by a number of studies from several different laboratories (4, 14, 16, 20). To gain some insights into the possible role of cell-cell communication in this process, we characterized the pattern of dye coupling and ionic coupling in all of the major extraembryonic tissues of the 7.5-d mouse embryo. Results from our present study showed that the extraembryonic tissues are segregated into several distinct communication compartments, and in conjunction with our previous results (7), indicate that the developing embryo is completely isolated from the bulk of the extraembryonic components of the mouse conceptus.

## Materials and Methods

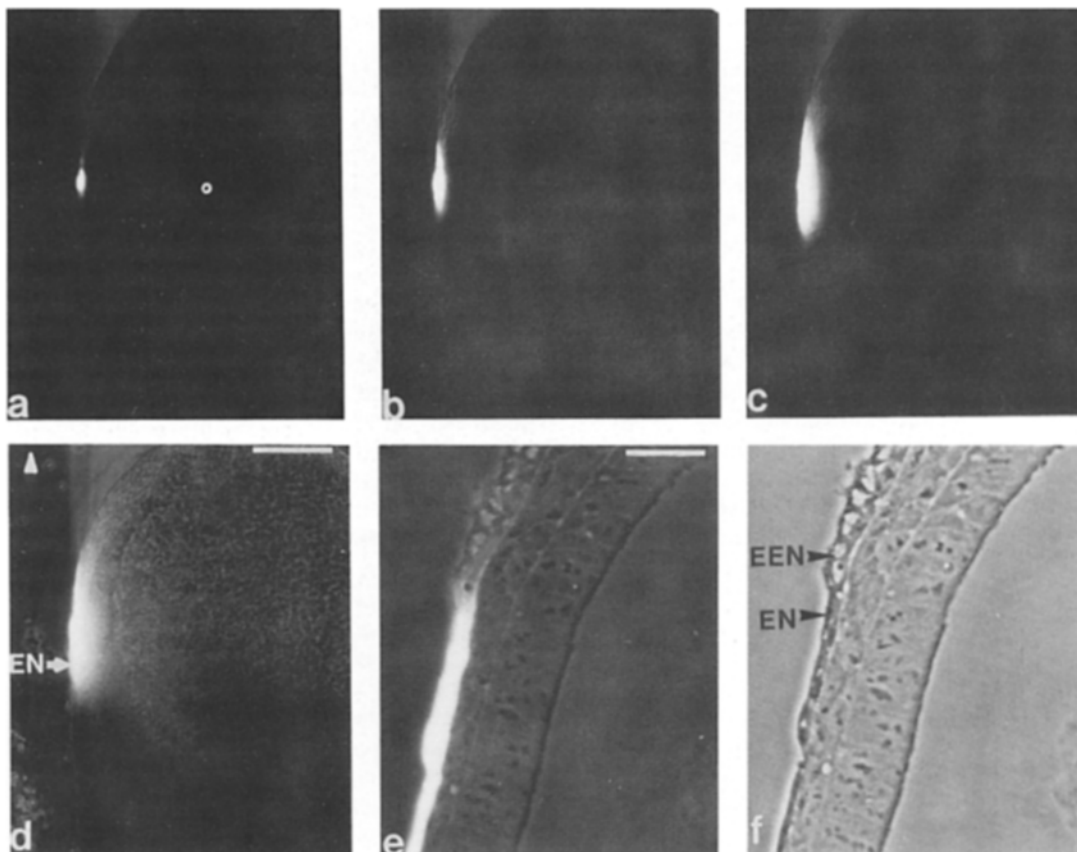
Procedures for embryo collection, intracellular Lucifer yellow injection, ionic coupling measurements, and histological analysis of the 7.5-d mouse embryo have been described previously by us (7). Impalements were made in extraembryonic ectoderm (EEC), visceral extraembryonic endoderm (EEN), visceral embryonic endoderm (EN), and in the central and peripheral regions of the ectoplacental cone (EPC). After the removal of elec-

trodes, each embryo was fixed in buffered formaldehyde, embedded in Spurr's resin, followed by serial sectioning, and examination of the intracellular fluorescence distribution by light microscopy.

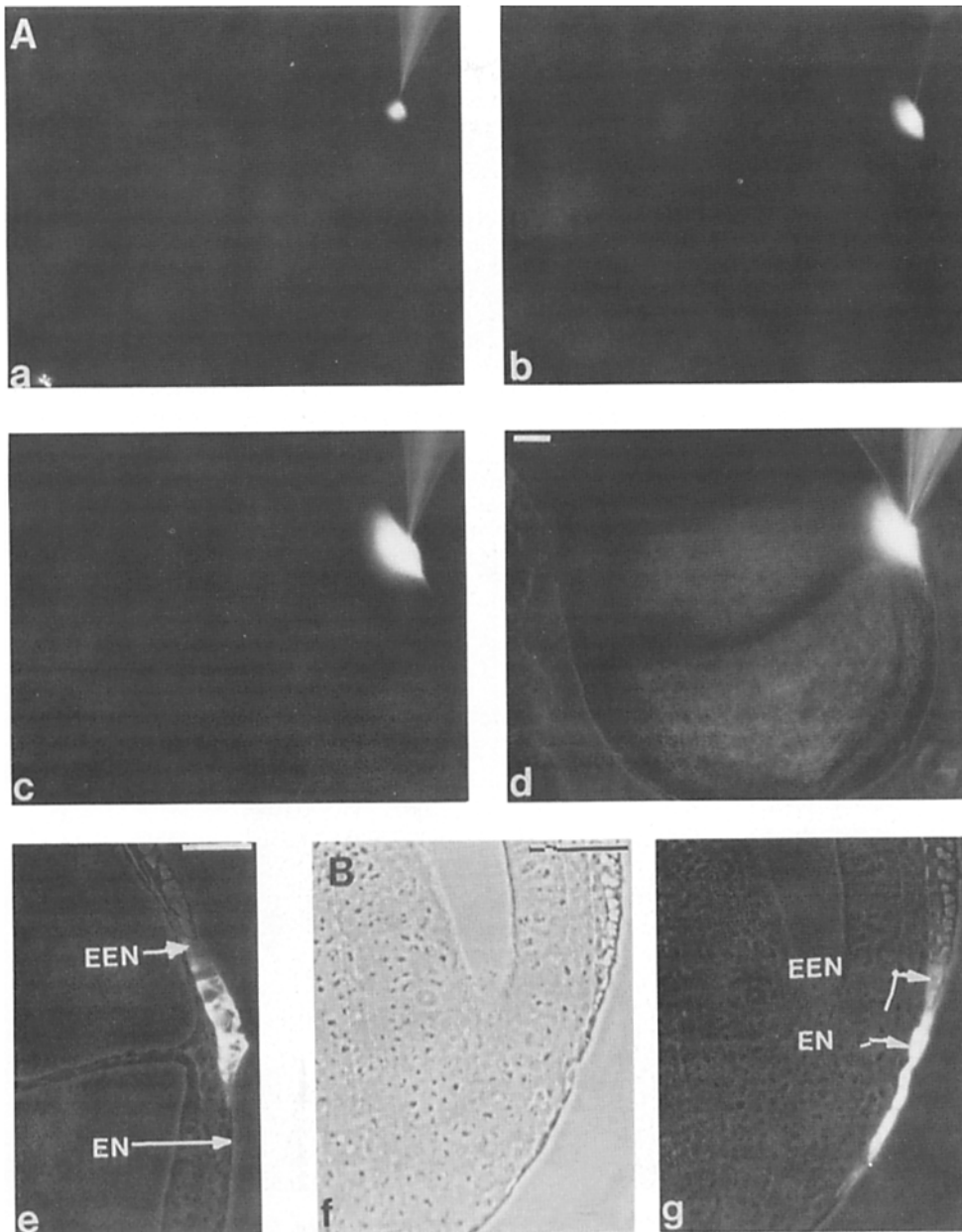
Dye injections were carried out with 1.0% Lucifer yellow ( $K^+$  or  $Li^+$  salts)-filled glass microelectrodes using continuous current pulses of 2–10 nA of 0.5 s duration once per second. The pattern of dye spread was recorded photographically at intervals during the course of injection. For monitoring ionic coupling, microelectrodes were filled with 1%  $K^+$ -Lucifer yellow in 50 mM KCl and Lucifer yellow was coinjected to facilitate the identification of the impalement sites in the subsequent histological analysis (7). Note that in all cases, the egg cylinder cavity was surgically opened to ensure that any ionic coupling detected was not an artifact arising from the presence of a permeability seal. No attempts were made to quantify the coupling efficiency (i.e.,  $V_2/V_1$ ) given the complex geometry of the embryo, and possible variations in nonjunctional membrane resistance. Nevertheless, it was possible to distinguish between qualitative differences in ionic coupling efficiency using the following criteria. Thus, when electrodes were placed, even on opposite sides of the embryo, and a voltage deflection of  $\sim 2$  mV was observed with 5–10 nA current pulses, it was considered strong ionic coupling. In contrast, a voltage deflection of  $< 2$  mV, obtained with closely juxtaposed microelectrodes using current pulses of up to 20 nA, was considered weak ionic coupling. Finally, in instances where voltage deflection could not be detected with 20-nA current pulses even with electrodes in close proximity, it was considered as indicative of no ionic coupling. This was observed with impalements between the EEN and all other extraembryonic tissues.

## Results

To characterize gap junctional communication in extraem-



**Figure 1.** Lucifer yellow injection in the visceral embryonic endoderm. A microelectrode was inserted into a visceral EN cell bordering the visceral EEN. Dark field fluorescence images at 30 s (*a*), 2 min (*b*), and 6 min (*c*), and phase-fluorescence image (*c*) at 7 min after the start of injection revealed highly asymmetric dye movement. Thick section histology in *f* and *e* shows that the dye was present only in the EN cells and appeared to have preferentially spread away from the EEN. Note that no fluorescence is detected in cells of the adjacent EEN (distinguishable by their columnar shape and highly vacuolated cytoplasm). Arrowhead in *d* indicates embryonic (antimesometrial) tip of the egg cylinder. Bars, 50  $\mu$ m.



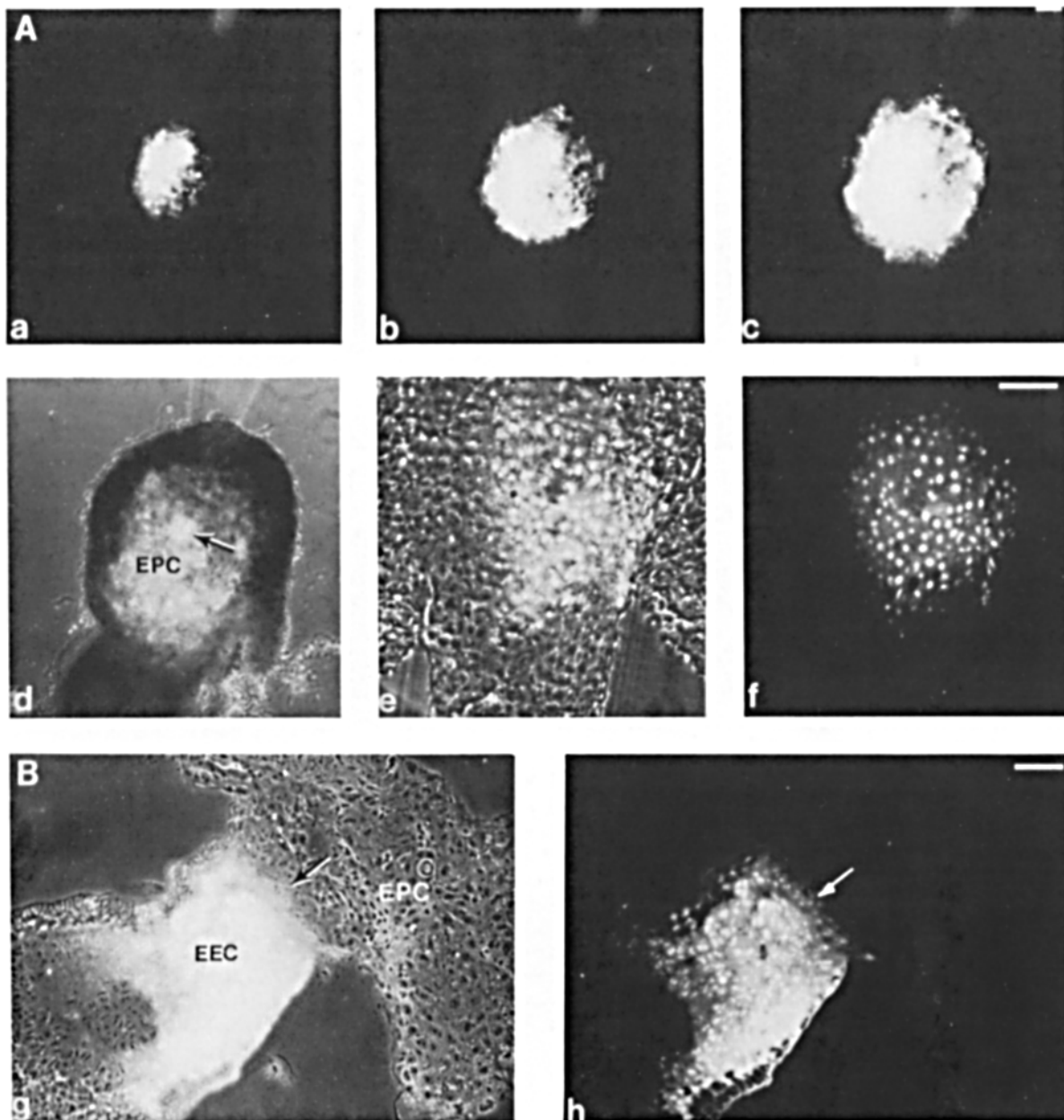
**Figure 2.** Lucifer yellow injection in the visceral EEN. **A**, Restriction in dye coupling between the visceral EEN and EN. The time course of dye spread after microelectrode impalement into an extraembryonic endodermal cell is illustrated by the dark field fluorescence images in *a-c* corresponding to 30 s, 2 min, and 7 min, and by the phase-fluorescence image in *d* corresponding to 8 min after the start of injection. The thick section shown in phase-fluorescence in *e* revealed that the Lucifer yellow is predominantly in the EEN. Note the very faint fluorescence in a few cells in the EN layer in *e*, thereby indicating a very low level of dye coupling at the EN/EEN border. **B**, Partial restriction in dye coupling at the EN/EEN border. Phase (*f*) and phase-fluorescent (*g*) images of a thick section from a Lucifer yellow-injected embryo show the presence of dye predominantly in the EN cells. Note the presence of two EEN cells at the EN/EEN border that exhibit faint fluorescence, thus indicating a low level of dye coupling between the EN and EEN.

embryonic tissues of the 7.5-d mouse embryo, we carried out Lucifer yellow injections and directly monitored the extent of dye spread in live specimens and also in histological sections of each dye injected embryo. For such studies, intracellular impalements were carried out in all of the major extraembryonic tissues. This included the visceral EEN, EPC, the EEC, and also the visceral EN. In addition, we also carried out parallel impalements to monitor ionic coupling, which should allow the detection of even low levels of coupling not detectable by dye coupling experiments. Below, we describe first the results of the dye injection experiments, and then these obtained in the ionic coupling studies.

**Partial Restriction in Dye Coupling between the Visceral EN and the Visceral EEN**

To examine gap junctional communication between the vis-

ceral EN and visceral EEN, dye injections were carried out with impalements at regions where these two tissues meet. The results of a series of such experiments are illustrated in Figs. 1 and 2. Impalements into the EN typically show good dye coupling, with the injected dye preferentially spreading away from the EN/EEN border (Fig. 1, *a-d*). Histological analysis of Spurr's sections confirm that almost all the Lucifer yellow is localized in the EN cells (Fig. 1, *e* and *f*; Fig. 2 *B*). These results indicate that the EN cells constitute a communication compartment. Note, however, very faint fluorescence was also frequently observed in a few EEN cells that are immediately adjacent to the EN/EEN border (Fig. 1, *e* and *f*; Fig. 2 *B*), suggesting that there is a low level of dye-coupling between the EN and EEN. Dye injections into the EEN yielded similar results (Fig. 2 *A*). The injected dye was predominantly localized in cells of the EEN, with only a very faint trace of dye detected in the immediately adjacent



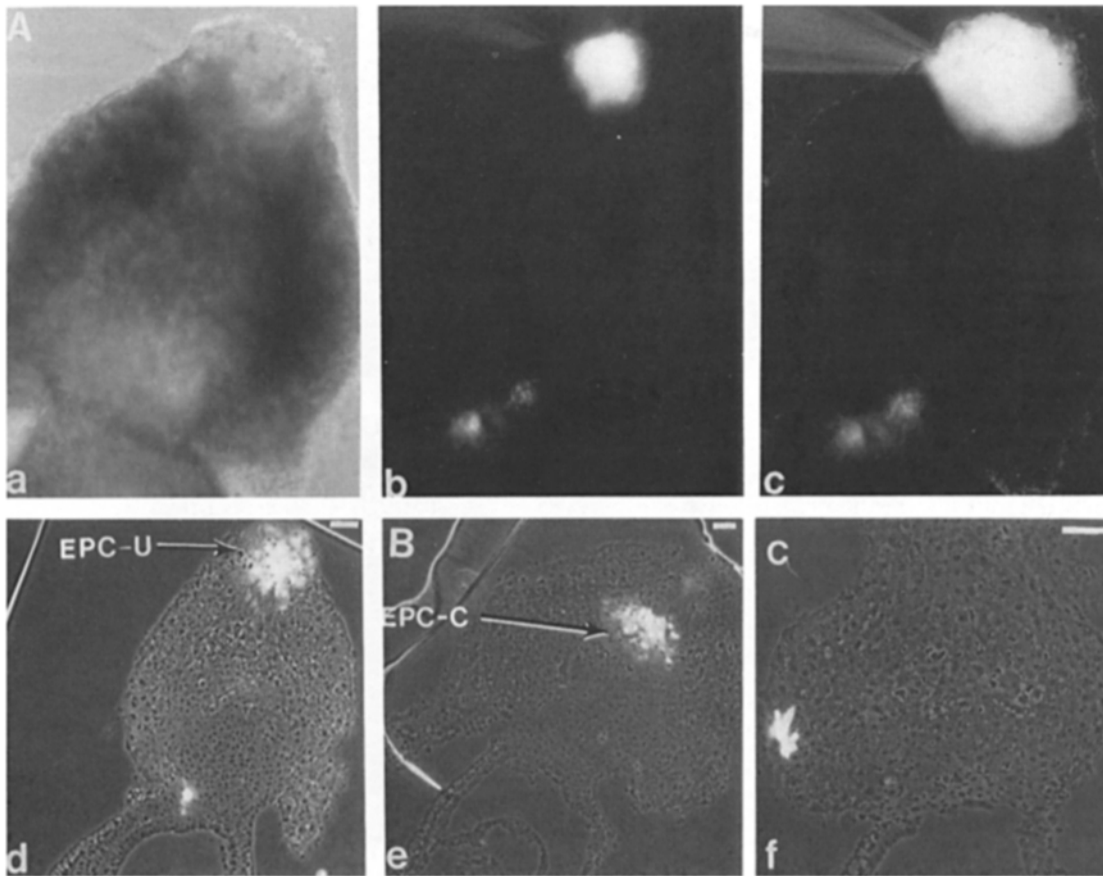
**Figure 3.** Lucifer yellow injection in the EPC and EEC. *A*, Restricted dye spread in the EPC-C. Lucifer yellow injection was carried out with an intracellular impalement at the central region of the EPC (EPC-C; arrow in *d*). Dark field-fluorescence images at 2 min (*a*), 5 min (*b*) and 8 min (*c*) after the start of injection revealed extensive dye spread that appeared to be confined to the central core of the EPC. This distribution was subsequently confirmed by thick section analysis as shown in *e* (phase-fluorescence) and *f* (dark field fluorescence). *B*, Restriction in dye coupling between the EEC and EPC. Thick section analysis of an embryo injected with Lucifer yellow (for 12 min) in the EEC revealed intense fluorescence in the EEC cells, but no dye spread to the adjacent EPC across the EEC/EPC border (arrow). Bars, 50  $\mu$ m.

EN cells (see Fig. 2 *e*). Based on these results, we conclude that the EN and EEN are each a separate communication compartment, although the boundary separating these two tissues is only partially restricted in dye coupling. This represents the only partial dye restriction border detected in the extraembryonic regions of the 7.5-d mouse embryo.

#### **Communication Compartments in the EPC and EEC**

In the central region of the ectoplacental cone (EPC-C), injection of Lucifer yellow revealed extensive dye spread, but with the dye being confined to the central core of the EPC

(Fig. 3 *A*). Thus even with prolonged injections in the EPC-C, Lucifer yellow was not observed in the peripheral regions of the EPC or in the EEC (for example see Fig. 3 *A*). Similarly, reciprocal injection of Lucifer yellow into the EEC exhibited extensive dye spread that was confined exclusively in the EEC, with no dye spread to the EPC detected (see Fig. 3 *B*). In the example of Fig. 3 *B*, Lucifer yellow filled a large number of cells in the EEC such that a distinct boundary was delineated between the highly fluorescent EEC cells and the nonfluorescent EPC cells. Other impalements into the peripheral regions of the EPC also showed a similar absence



**Figure 4.** Dye coupling in other regions of the EPC. *A*, Restriction in dye coupling in the upper peripheral region of the EPC (EPC-U). An impalement into the upper peripheral region of the EPC (*a*) revealed a pattern of limited Lucifer yellow spread, with dye filling only a cluster of cells in the upper region of the EPC as shown by dark field fluorescence images at 2 min (*b*), and 10 min (*c*) after the start of injection. Even after 13 min of injection, no further dye movement is observed. Thick section analysis confirmed the presence of fluorescent dye in a distinct cluster of cells in the upper peripheral EPC (*d*). *B*, Restriction in dye coupling at the EPC-C/EPC-U border. Lucifer yellow was injected for 4 min into a cell at the EPC-C, very near the EPC-U. Histological analysis revealed that the fluorescent dye is present in a distinct cluster of EPC-C cells delineating a semicircular pattern, as can be seen in the phase-fluorescence thick section image in *e*. The faint fluorescence in the very distal region of the EPC-C is because of a previous impalement. *C*, Limited dye coupling in the lateral peripheral regions of the EPC. Impalement and Lucifer yellow injection into the EPC-L resulted in dye spread only to a small cluster of cells (even after 8 min of injection) as illustrated in the thick section phase-fluorescence image in *f*. Bars, 50  $\mu$ m.

of dye spread to the EEC. These results show that the EPC-C and EEC each constitute a separate communication compartment.

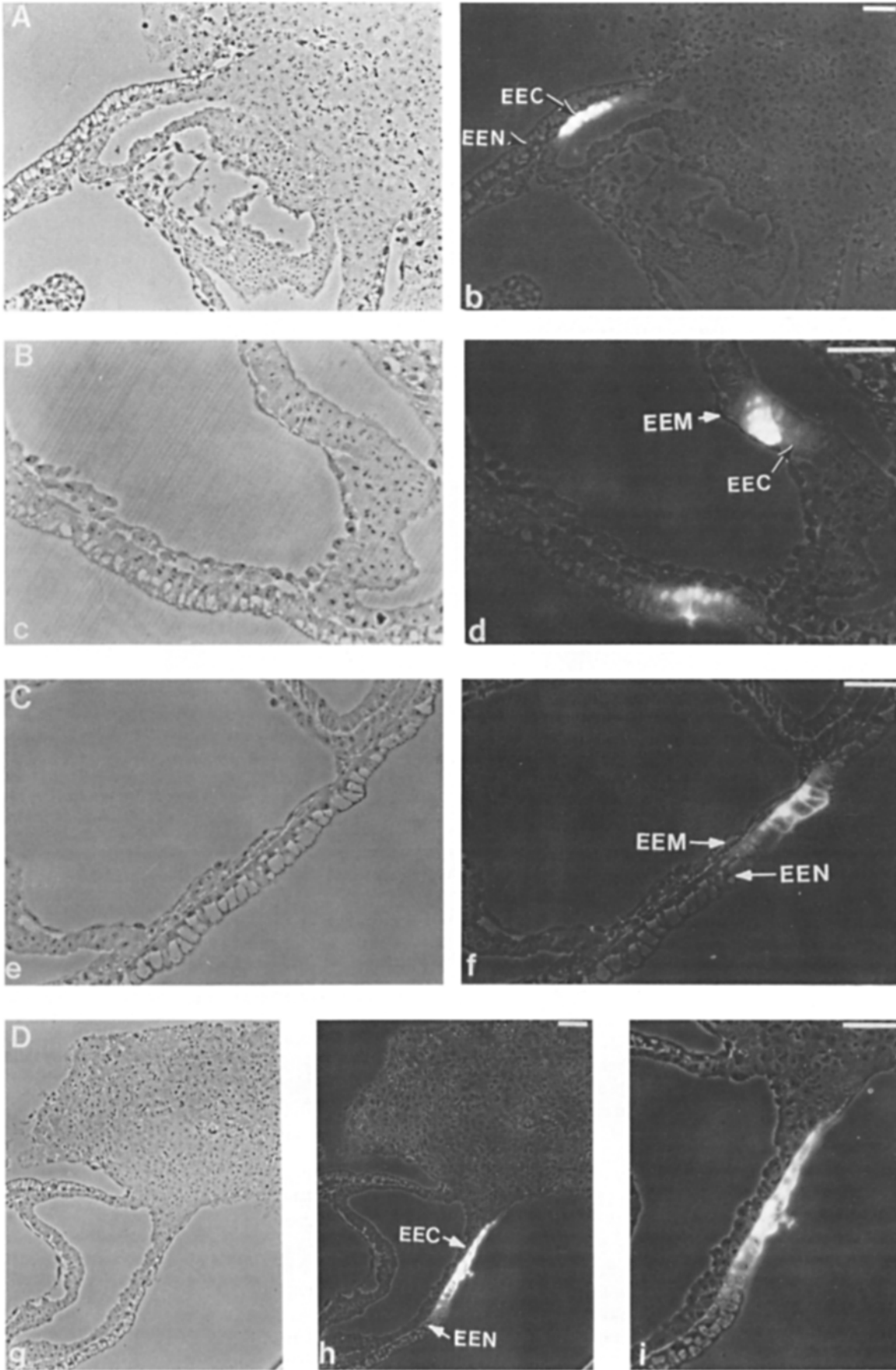
Impalements in the upper peripheral regions of the EPC (EPC-U) revealed extensive dye spread which delineated a distinct subdomain of cells which defined another compartment in the EPC (Fig. 4 *A*). This pattern was observed even with prolonged injections of Lucifer yellow. That cells in this compartment are not dye coupled to the EPC-C was confirmed by examining sections of such dye injected embryos. Thus, in the embryo shown in Fig. 4 *A*, Lucifer yellow was only found in cells of the EPC-U but not in the lateral peripheral (EPC-L) or in the central regions of the EPC (see Fig. 4 *d*). Similarly, impalements carried out in the EPC-C revealed no dye spread to the EPC-U domain. In the example of Fig. 4 *B*, Lucifer yellow injected into cells in the EPC-C resulted in a cluster of dye-filled cells that immediately abutted cells in the EPC-U that were completely devoid of any fluorescent dye.

In contrast to the EPC-U and EPC-C, cells of the lateral

peripheral regions (EPC-L) exhibited very poor dye coupling. Dye injections into the EPC-L usually resulted in dye spread to only a single cell or a very small cluster of 2–4 cells (Fig. 4 *C*). Such dye-filled clusters become intensely fluorescent upon further dye injection, but without any dye spread to other neighboring cells. These results indicate that the peripheral regions of the EPC are segregated into two domains: an EPC-L zone that exhibits very poor dye coupling, and an EPC-U that contains cells that are well coupled.

#### **Complete restriction in dye coupling between EEC, EN, and Extraembryonic Mesoderm (EEM)**

Impalements and injections of Lucifer yellow were also carried out to examine dye coupling between the EEC, visceral EEN, and EEM. These three tissues show extensive areas of cell–cell contact (see Fig. 10). The EEN is a single cell layer immediately adjacent to the EEM and EEC, with the EEM juxtaposed to the EEC only at the upper portion of the exocoelomic cavity. Impalements in the EEC or EEN were relatively straightforward, and usually resulted in extensive dye



**Table I. Dye Coupling in the Extraembryonic Regions of the 7.5-d Mouse Embryo**

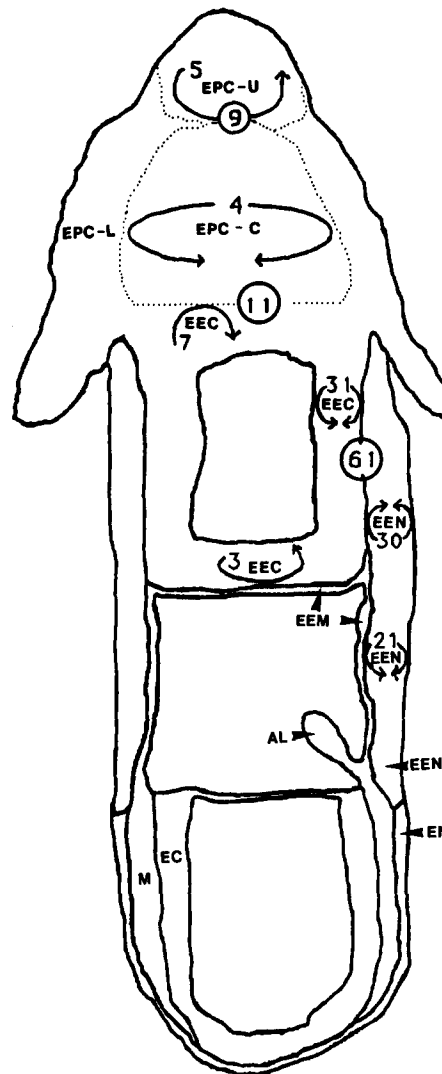
Region of dye fill*	No. of embryos analyzed
EEC	41
Visceral EEN	53
EPC	9
EEM	1
Visceral EN	3
EEC and EEN†	2
EN and EEN‡	5

\* Lucifer yellow was used for all injections. Each embryo was embedded and sectioned to ascertain the site of impalement and the extent of dye spread.  
 † The dye movement observed beyond the EEC/EEN border is likely because of accidental displacement of the electrode, since the Lucifer yellow in both embryos was observed to be localized predominantly in one cell type.  
 ‡ Dye injected in either the EN or EEN spreads asymmetrically, predominantly away from the border delineating these two cell types. However, as a small amount of dye is always seen to cross the border, it is likely that the EN/EEN border is only partially restricted in dye coupling.

spread. In contrast, all of our attempts at impalements into the EEM, with the exception of the allantois, were not successful because of the very thin single cell-layered architecture. Hence restrictions in coupling at the EEM boundaries were by necessity, deduced from the results of impalements in the EEC or EEN. Extensive dye spread was observed within the EEC or EEN, but no dye coupling was detected between these two tissues, nor between these two tissues and the EEM. Four examples of impalements in these regions are illustrated in Fig. 5. In each case, an examination of sections from the dye-injected embryos revealed that the Lucifer yellow was completely restricted to within either the EEC (for example, see Fig. 5, A and B) or EEN (for example, see Fig. 5, C and D).

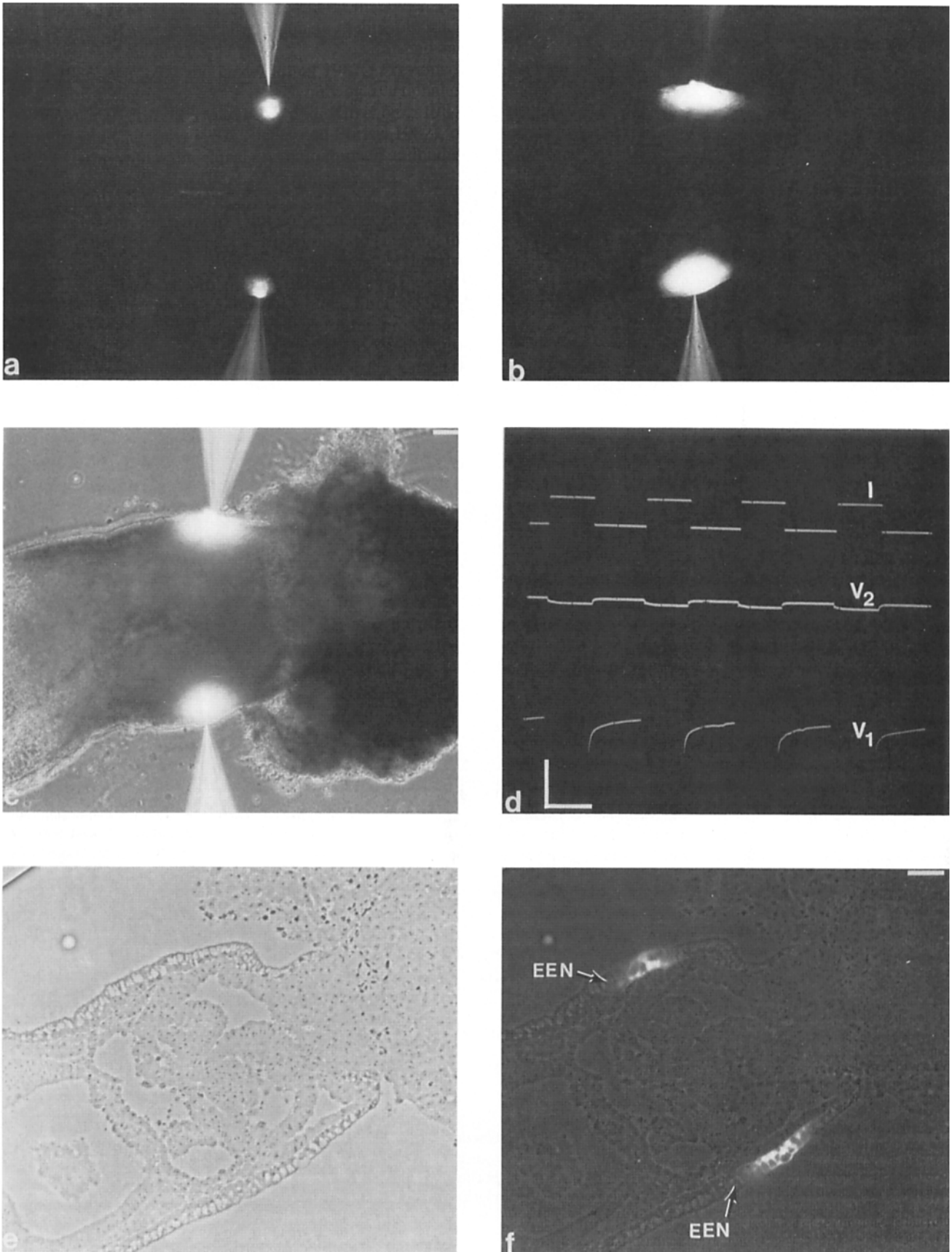
**Summary of Dye Coupling in the Extraembryonic Tissues**

Results from all the dye injection experiments are summarized in Table I. Except for cells at the EEN/EN border, dye spread was almost always limited to cells of the same tissue type. This was observed in 107 of 109 impalements. In the two exceptions, Lucifer yellow was found predominantly in the EEC in one case, and in the EEN in the other. This apparent dye spread is likely a result of accidental microelectrode movement during dye injection. The precise spatial distribution of the dye-delineated boundaries is diagrammatically represented in Fig. 6. Thus, for example, of the 41 injections performed in the EEC, seven were near the central EPC region and exhibited restriction in dye spread at the EEC/EPC



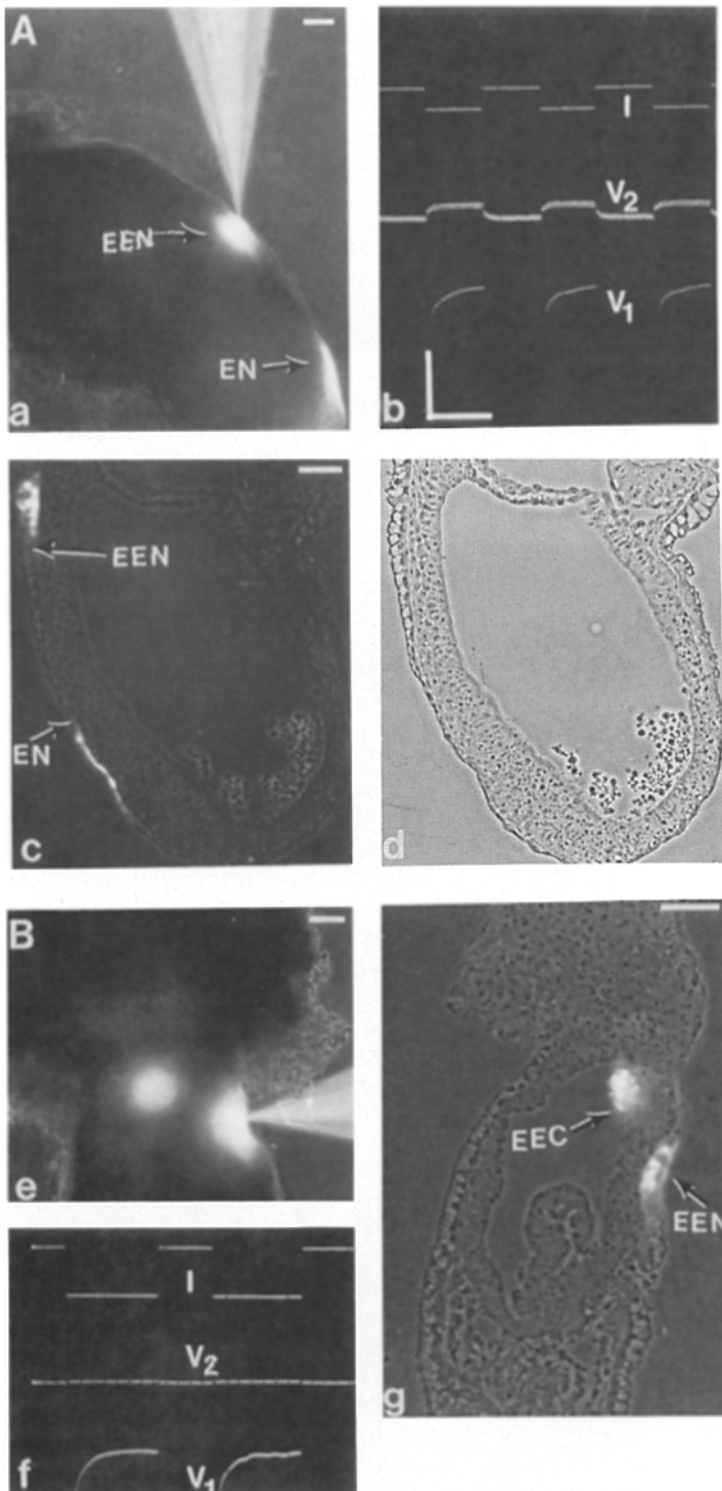
**Figure 6.** Summary of dye coupling data obtained in the 7.5-d mouse embryo. Boundaries delineated by the solid and dotted lines correspond to the positions of restricted Lucifer yellow dye spread observed in the 7.5-d mouse embryo. Thus, areas outlined by the dotted lines depict communication compartments observed within the ectoplacental cone, while areas included within solid lines correspond to communication compartments that also happen to coincide with known tissue types as indicated. The numbers bracketed by the curved arrows denote the number of embryos in which the spread of Lucifer yellow was observed to be restricted to that particular compartment. The circled numbers denote the total number of impalements in which a particular communication restriction boundary was observed with dye injections from either side of the border. AL, allantois; EC, embryonic ectoderm; and M, mesoderm.

**Figure 5.** Dye coupling in the EEC and EEN. A, Extraembryonic ectoderm is segregated from the visceral EEN. In this embryo, Lucifer yellow was injected for 4 min into the EEC. Phase (a) and phase-fluorescence (b) images of a thick section confirm the presence of fluorescent dye only in the EEC cells, with no dye detected in the neighboring EEN cells. B, Extraembryonic ectoderm is segregated from the extraembryonic mesoderm. Phase (c) and phase-fluorescence (d) images of a section from a Lucifer yellow-injected embryo (3 min injection time) revealed the presence of fluorescent EEC cells (black and white arrow), but no fluorescence in the neighboring EEM (white arrow). The second area of fluorescence in the lower portion of the embryo is because of another impalement in the EEN. C, Visceral EEN is segregated from the EEM. Phase (e) and phase-fluorescence (f) images of a section from a Lucifer yellow-injected (3 min duration) embryo show that the Lucifer yellow was present only in the EEN and not in the neighboring EEM layer. D, Dye spread in the visceral EEN is restricted from the EEC. Phase (g) and phase-fluorescence (h and i) images of a section from a Lucifer yellow-injected embryo show the presence of fluorescence in cells of the EEN, but not in the adjacent EEC. Bars, 50 μm.



**Figure 7.** Ionic coupling between cells of the EEN. Ionic coupling was monitored between two visceral extraembryonic endodermal cells on opposite sides of a 7.5-d embryo using Lucifer yellow-KCl filled microelectrodes. Dark field images at 1 min (a) and 2 min (b) after the start of impalements are shown. The precise positions of the two microelectrodes in the live specimen are illustrated by the fluorescence distribution observed in the phase-fluorescence image in c (photographed after 4 min of injection). The endodermal location of both impale-





**Figure 8.** Ionic coupling between the visceral EEN and the visceral EN/EEC. **A**, Ionic coupling with the visceral EN. Two Lucifer yellow-KCl filled microelectrodes were impaled into a 7.5-d embryo as shown in the phase-fluorescence image in *a* (5 min after the start of impalement). Thick section analysis demonstrates that these impalements were located in the EN and EEN, respectively (*c* and *d*). When current was pulsed in one microelectrode ( $V_1$ ), a simultaneous voltage deflection was detected in the second microelectrode ( $V_2$ ), thus indicating the presence of ionic coupling between these two cell types. (*b*) Vertical bar, 20 nA/10 mV; and horizontal bar, 500 ms. Bars, (*a* and *c*) 50  $\mu$ m. **B**, Ionic coupling with the EEC. No ionic coupling was detected between cells in the EEC and EEN. In this example, the two sites of impalements can be observed in the phase-fluorescence image in *e* (recorded 6 min after the start of impalements). The microelectrode in the EEN is clearly visible (*right*), while the microelectrode (*left*) in the EEC is out of the plane of focus. The precise location of these two impalement sites was confirmed by thick section analysis that showed the presence of fluorescence (*g*) in the EEN and EEC, respectively. Note the absence of voltage deflection in the alternate electrode as current is passed from either impalement site (*f*). Bars, (*e* and *g*) 50  $\mu$ m; *f*, see bar in *b*.

boundary, 31 were in the EEC at the vicinity of the EEN and exhibited no dye spread to the EEN, while three were in the EEC in close proximity to the EEM, revealing a complete absence of dye spread to the EEM. Similarly, impalements

in the EEN in 30 embryos revealed no dye coupling with the EEC, while EEN impalements in another 21 embryos revealed no dye coupling with the EEM. The sum of all impalements which delineated a particular boundary of re-

ments was confirmed by the finding of fluorescence only in cells of the EEN layer as observed in the phase (*e*) and phase-fluorescence (*f*) images of a thick section. The presence of ionic coupling between the two impalement sites is indicated by the oscilloscope trace shown in *d*;  $V_1$  is the voltage recorded from the current (I) passing electrode, and  $V_2$ , the simultaneous voltage deflection detected in the second electrode. Bars, (*a-c*, *e* and *f*) 50  $\mu$ m. (*d*) Vertical bar, 20 mV/20 nA; and horizontal bar, 500 ms.

**Table II. Ionic Coupling Measurements in the Extraembryonic Regions of the 7.5-d Mouse Embryo**

Site of impalements*	Ionically coupled	Not coupled
EEC and EPC-C	21 (10)‡	—
EEN and EPC	—	11
EEN and EEC	—	7
EEN and EN	8	—
EPC-C and EPC-L/U	9 (6)‡	—
EEC and EPC-L/U	—	4
EEN and EEN	5	—
EEC and EEC	4	—
EPC-C and EPC-C	2	—

\* A small amount of Lucifer yellow was injected at each impalement site to facilitate subsequent histological analysis for determining the precise point of impalement.

‡ Figures in parentheses denote the subset of embryos in which the ionic coupling was at low levels (see Materials and Methods section).

stricted dye spread is represented in circles at the appropriate positions in Fig. 6. This summation included injections carried out from either side of a border. Thus, impalements in 61 embryos demonstrated restriction in dye coupling between the EEN and EEC, 11 showed restriction between the EEC and EPC, and nine showed restriction between the EPC-C and EPC-U.

### Ionic Coupling Analyses

Parallel impalements to monitor ionic coupling revealed that most of the extraembryonic tissues were well coupled to each other. With impalements into the EEN (Fig. 7), ionic coupling was easily detected as indicated by the finding of simultaneous voltage deflection in both electrodes when current was pulsed from either electrode (Fig. 7*d*). In the example of Fig. 7, ionic coupling was detected between EEN cells on opposite sides of an embryo. The two impalement sites were clearly visualized by the Lucifer yellow injected into the impaled cells (Fig. 7, *a-c*) and the EEN location was confirmed histologically by the finding of fluorescent dye only in the endodermal cells (Fig. 7, *e* and *f*). Strong ionic coupling was also observed between the EEN and EN cells (Fig. 8*A*). Ionic coupling also was detected even with electrodes placed on opposite sides of the embryo. However, no ionic coupling was ever observed between the EEN and EEC, even with impalements that were closely juxtaposed (Fig. 8*B*). Similarly, 11 impalements in the EEN and EPC also showed no ionic coupling between these two tissues (Table II).

With impalements in the EPC, a more complex pattern was observed. Between the central and peripheral regions of the EPC a low level of ionic coupling was found (Fig. 9, *A*), while a high level of ionic coupling was observed between the central EPC and the EEC (Fig. 9, *B*). In contrast, no ionic coupling could be detected between the EEC and the peripheral regions of the EPC. Given that the EEC showed strong ionic coupling with the EPC-C, and the EPC-C exhibited a low level of ionic coupling with the peripheral EPC, it is expected that the EEC should exhibit a very low level of ionic coupling with the peripheral EPC. However, it is likely that the greater distance which usually separated impalements in these two tissues prevented the detection of ionic coupling. In comparison, impalements in the EEN and EEC, even those immediately juxtaposed, did not show any

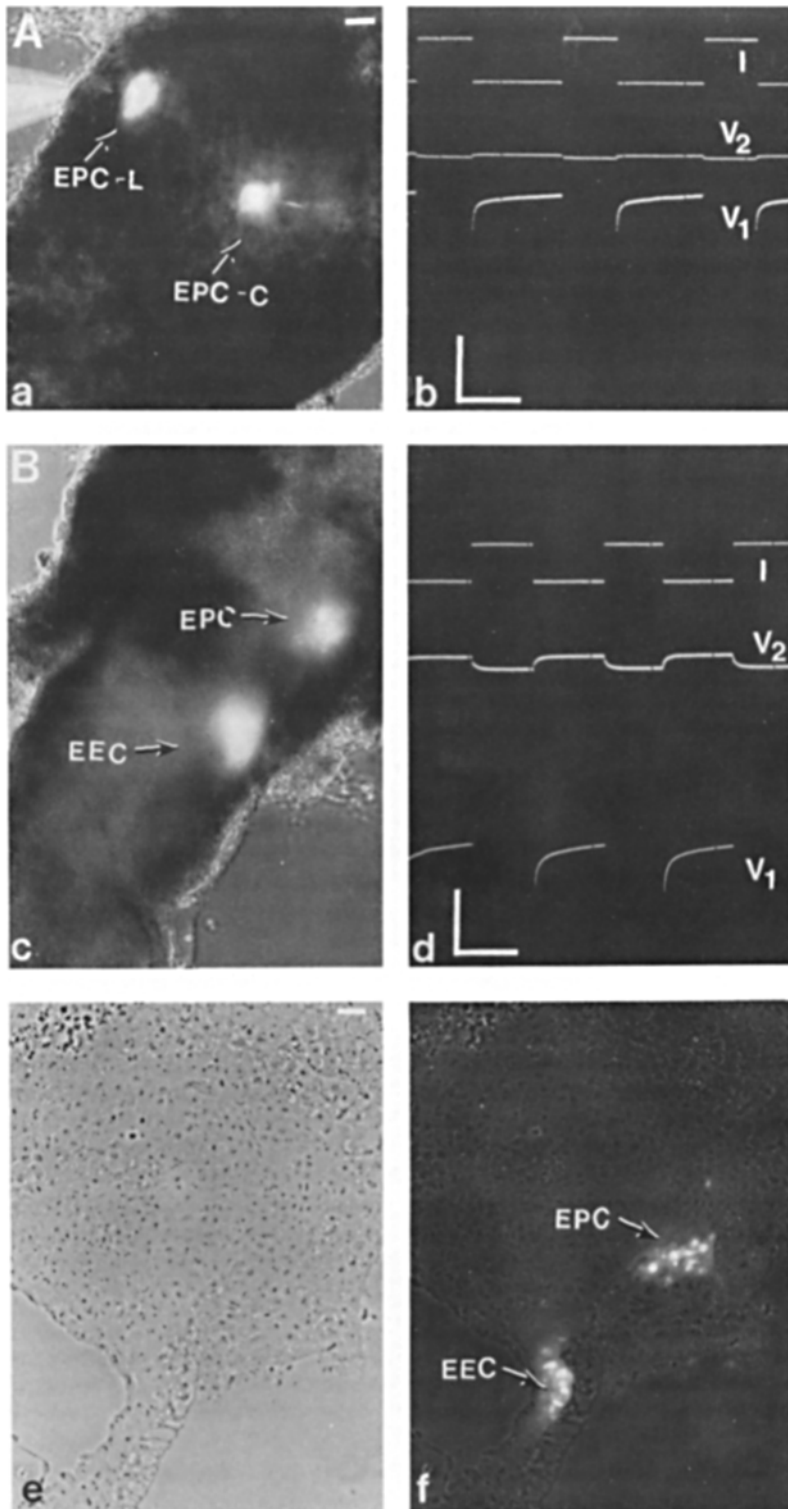
detectable coupling, while impalements spaced apart at great distances in the EEN and EN and within the EEN consistently exhibited ionic coupling.

The results of these ionic coupling studies are summarized in Table II. They show that only the EEN is not ionically coupled with the other two major extraembryonic tissues, the EEC and EPC. However, note that the EEN is ionically coupled to itself and to cells in the EN layer. Given that our previous study demonstrated that the EN cells are ionically coupled to cells in the embryonic mesoderm and ectoderm layer, the EEN must also be coupled to cells in these two embryonic germ layers. Thus, overall, the ionic coupling data indicate that the embryo as a whole is organized into two distinct ionic coupling domains, one encompassing the embryo proper and the extraembryonic endoderm, and another encompassing all of the remaining extraembryonic tissues (see Fig. 10). Note that as discussed above, the ionic coupling status of the EEM remains unknown.

### Discussion

The results of our investigation show that the extraembryonic tissues of the 7.5-d mouse conceptus are segregated into communication compartments. With Lucifer yellow injections, the visceral EEN, visceral EN, EEC, the EEM, and the central and peripheral regions of the EPC each appeared as separate communication compartments. Previously, similar experiments showed that each of the germ layers in the embryo proper also correspond to individual communication compartments (7). Thus, the cumulative results from these two studies demonstrate the presence of nine major dye-delineated communication compartments in the 7.5-d mouse embryo (see Figs. 6 and 10). Of particular significance is the finding that most of these compartments were not completely isolated from one another as they remained ionically coupled. This effectively subdivides the 7.5-d mouse conceptus into two larger domains (see Fig. 10), which are completely isolated from one another. Interestingly, cells in these two domains are also derived from separate lineages, with one domain consisting of the EC, mesoderm (M), EN, and EEN being of ICM origin, while the other domain consisting of the EEC and EPC belonging to the trophoctoderm lineage (4). These findings differ somewhat from the earlier studies of Lo and Gilula (12) which also showed that the ICM and trophoctoderm of mouse blastocysts implanted *in vitro* were organized as separate communication compartments, but the latter compartments were ionically coupled. This difference could easily arise from the fact that embryos implanted *in vitro* may not maintain the appropriate spatial organization and architecture required to establish the isolation that is characteristic of these two domains. It is also possible that there is a development dependent progression in gap junctional communication restriction that is lineage specific and results first in the loss of dye coupling and is then followed by the disappearance of ionic coupling.

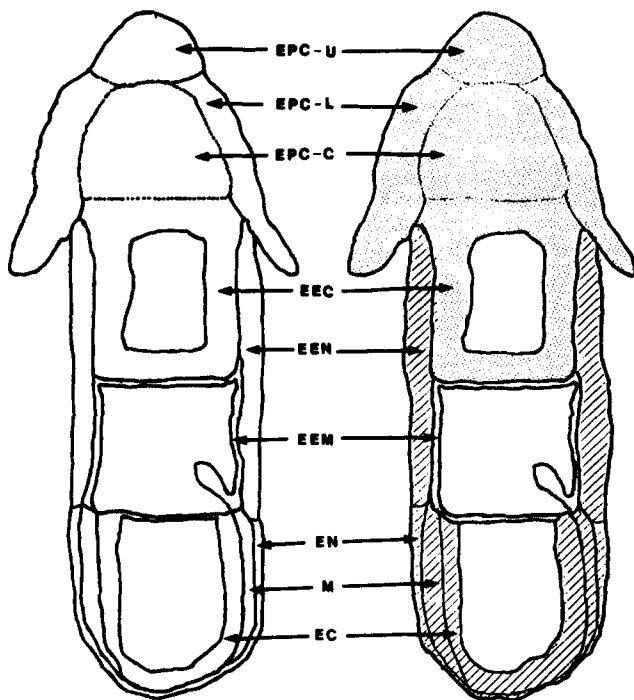
It is also interesting to note that the three subcompartments observed in the EPC might be functionally relevant to the ongoing regionalization in this complex tissue. Thus, cells in the EPC-C are diploid, while those in the EPC-L are destined to endoreplicate and undergo giant cell transformation (5, 18). In contrast, in the EPC-U, also referred to as the "mesometrial end of the EPC" (9), spongiotrophoblast pre-



**Figure 9.** Ionic coupling in the ectoplacental cone and in the EEC. *A*, Ionic coupling between the central and peripheral regions of the EPC. Two impalements into the EPC-C and EPC-P regions of the ectoplacental cone are shown in the phase-fluorescence image in *a* (recorded 4 min after the start of impalement). A low level of ionic coupling was detected between these two impalement sites as illustrated by the oscilloscope trace in *b*. *B*, Ionic coupling between the EEC and the EPC. A phase-fluorescence (*d*) image (recorded at 5 min after the start of impalement) show two impalements, which by thick section analysis was determined to be in the EEC and EPC (*e* and *f*). Ionic coupling between these two cell types was demonstrated by the finding of a simultaneous voltage deflection when current was pulsed from either microelectrode (*d*). Bars, (*a* and *e*) 50  $\mu\text{m}$ . (*b* and *d*) Vertical bar, 20 nA/10 mV; and horizontal bar, 500 ms.

cursors are known to be present. Further consistent with gap junctional coupling playing a role in the ongoing diversification of cell lineages is the finding that the two communication compartments corresponding to the EEC and central EPC consist of cells that are morphologically indistinguishable but nevertheless exhibit very different protein synthetic patterns (6). The EEC has been proposed to contain stem cells or precursors for the central EPC, which in turn may differentiate into peripheral giant cells (18).

In light of these observations, and in conjunction with our previous analysis of the embryo proper, we suggest that this process of communication compartmentation may play an important role in specifying the global organization of positional information in the early mouse embryo. Consistent with this hypothesis is the finding of no detectable cell-cell coupling between the embryo proper and the extraembryonic tissues destined to form the placenta. Thus, if communication compartments provide the context for the formation



**Figure 10.** Nine major dye-delineated communication compartments and their integration into two ionically coupled domains. Each of the nine labeled regions in the egg cylinder illustrated on the left represents a communication compartment observed by Lucifer yellow injection. Most of these communication compartments maintain some level of ionic coupling across compartment borders. However, the absence of any ionic coupling between the EEC and EEN effectively segregates the embryo into two domains, as indicated by the hatched and stippled regions in the egg cylinder on the right. Thus, the hatched region represents a domain of continuity between several dye-delineated communication compartments at the ionic coupling level (i.e., between the EC, M, EN, and EEN). The stippled area represents another such domain which includes the EEC and the different compartments in the EPC. Note that the EEM region is left unshaded as impalements were not obtained in this region.

or maintenance of "positional information" in the embryo proper, then it might be necessary to restrict gap junctional communication between the embryo proper from the placental tissues that are known to establish gap junctional contacts with the uterine epithelium (15, 19). We suggest that the maintenance of coupling between the embryonic germ layers and the visceral embryonic and extraembryonic endoderm may reflect the nutritional roles these endodermal cells likely play in supporting early embryogenesis. Cells in the EN and EEN are known to absorb nutrients from the maternal circulation and secrete various proteins and factors, including alpha fetoprotein (4). The low level of ionic coupling that continues to span across communication compartments, both in the extraembryonic and embryonic domains, may facilitate the global coordination of developmental and metabolic activities in the conceptus. The validity of this hypothesis may

be further evaluated in the future by examining gap junctional interactions between the peripheral EPC, especially between the EPC-U with the maternal tissue, as well as between the parietal endoderm and the surrounding maternal decidua.

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