

ELECTROPHYSIOLOGICAL EVIDENCE FOR LOW-RESISTANCE INTERCELLULAR JUNCTIONS IN THE EARLY CHICK EMBRYO

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ABSTRACT

Electrophysiological evidence is presented for the exchange of small ions directly between cells interiors, i.e. "electrical coupling," in the early chick embryo. Experiments with intracellular marking show that coupling is widespread, occurring between cells in the same tissue, e.g. ectoderm, notochord, neural plate, mesoderm, and Hensen's node, and between cells in different tissues, e.g. notochord to neural plate, notochord to neural tube, notochord to mesoderm. The coupling demonstrates the presence of specialized low-resistance intercellular junctions as found in other embryos and numerous adult tissues. The results are discussed in relation to recent electron microscopical studies of intercellular junctions in the early chick embryo. The function of the electrical coupling in embryogenesis remains unknown, but some possibilities are considered.

INTRODUCTION

Studies of the control of embryonic cell movement and differentiation have often emphasized the importance of interactions occurring at points of apparent cell contact (30), e.g. induction (7) and "contact inhibition" (1, 28). There are several ways by which apposed cells might influence each other, as short-range secretion (6), selective adhesion (25), or the direct exchange of substances between the cell interiors at specialized points of contact (14, 16, 26). An example of the third mechanism, known to occur widely in cleaving newt embryos (8) and in various adult tissues (2, 13), was recently demonstrated in the squid embryo by Potter et al. (16) who used electrophysiological techniques to follow the cell-to-cell movement of small ions. They showed that, during much of the developmental period and even after appreciable cellular differentiation, characteristic electrical changes were produced in a variety of cells tested when a pulse of current was supplied to the inside of the large yolk cell. As in other similar cases (2, 8, 13), this "electrical coupling" appears to depend upon the direct movement of small ions

between adjacent cell interiors via special low-resistance contacts. The interest in these junctions is enhanced by the possibility that they might also permit the direct exchange of molecules considerably larger than the small ions traced by the electrical methods (13, 16).

In the light of these studies and speculations it was of interest to investigate the distribution of electrical coupling in another embryo. The early chick embryo has certain technical advantages because it can be easily separated from the yolk and is then transparent so that areas of interest can be discerned for microelectrode impalements. In addition, its development has been well studied from the standpoints of morphology (20, 24, 27) and experimental embryology (15, 19, 29). It was of special interest to look for coupling between cells around Hensen's node, for this region is a focal point for early cellular interactions (29). Other regions of the embryo were studied, but in less detail. A preliminary report of this work appeared previously (23).

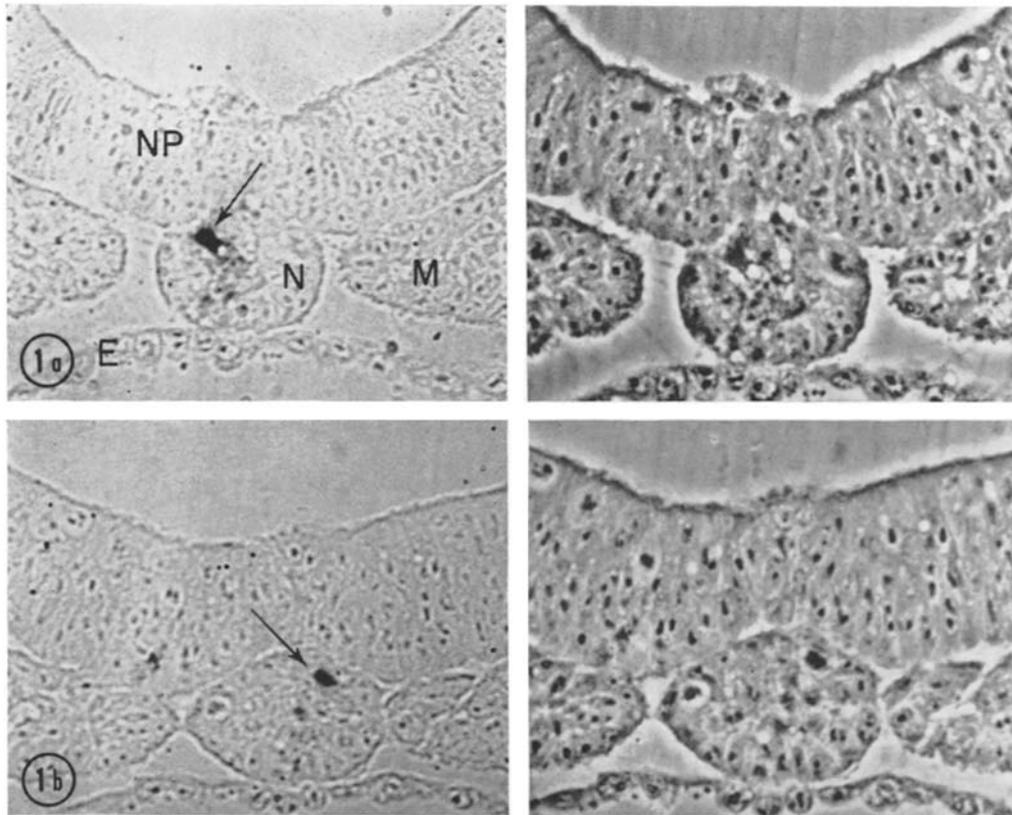


FIGURE 1 Arrows indicate two coupled notochord cells in an 11-somite embryo. The two cross-sections, *a* and *b*, were taken $50\ \mu$ apart and are shown on the left with bright-field, and on the right with phase-contrast optics. *N*, notochord; *M*, paraxial dorsal mesoderm; *NP*, neural plate; *E*, endoderm. Left, $\times 480$; right, $\times 550$.

MATERIALS AND METHODS

Fertilized White-Leghorn eggs were incubated at 39°C for 18–72 hr. The egg was broken into a dish containing Locke's solution (21) at room temperature (25°C). The blastodisc was cut away, placed ventral side up in a small plastic dish containing Locke's solution, and pinned with small tungsten needles to a layer of soft, transparent plastic (Sylgard from Dow Corning Corp., Midland, Mich.) covering the bottom of the dish. The electrical measurements and histological fixation were carried out with the blastodisc attached to the dish. The experiments were of relatively short duration, generally less than 2 hr. Most experiments were made at 25°C ; in a few experiments the Locke's solution was heated to 37°C before use, but no difference in results was found, except for a slight increase in resting potential.

Standard glass micropipettes (tips less than $0.5\ \mu$) were used for passing current and recording potential

changes. In most experiments the micropipettes were filled with a negatively charged, blue dye (Niagara sky blue: 6B, 3.5% by weight in aqueous solution).¹ The procedure for determining electrical coupling between two cells was to impale each with a micropipette. A rectangular current pulse (10^{-8} – 10^{-7} amp for 1 sec) was passed through one micropipette while any resulting changes in membrane potential were monitored by the second micropipette. Then the current pulse was repeated with the recording micropipette withdrawn, or advanced, to an extracellular position. Electrical coupling between the two cells was indicated by the appearance of an electrotonic potential change inside, but not outside, the second cell. When dye-filled pipettes were used, the impaled cells were marked by the electrophoretic deposition

¹ Alvarez, J., and E. J. Furshpan. Personal communication.

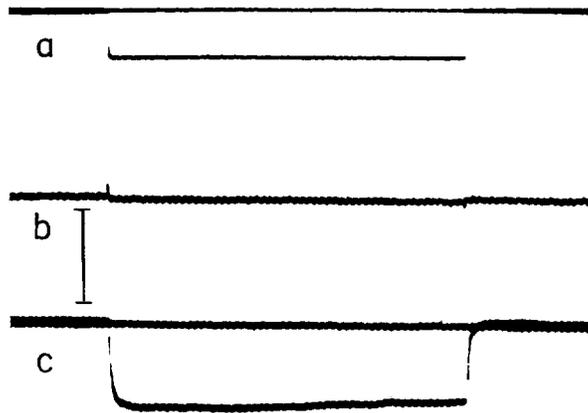


FIGURE 2 Electrical records for cells in Fig. 1 *a*, current pulse (3×10^{-8} A for 0.95 sec) in one notochord cell; *b*, potential just outside, and *c*, just inside, second notochord cell. Resting potential of second notochord cell, 6.4 mv. Voltage calibration for *b* and *c*, 5 mv.

of the dye inside them, and their positions were determined in histological sections.

Each micropipette was connected to a separate high impedance input amplifier and stimulus isolation unit so that either micropipette could be used for passing current or recording. Separate Ag-AgCl bath electrodes were used in the recording and current-passing circuits.

The excised blastodiscs were illuminated from below through a dark-field condenser and were observed with a dissecting stereomicroscope, except in one series of experiments made with a compound microscope ($\times 10$ objective).

Persistent difficulties were presented by the small size of the cells (less than 10μ diameter). It was not possible, for example, to impale a cell with more than one electrode to determine accurately its input resistance. Nor was it possible to keep an electrode in one cell while successively impaling other cells to determine the fall of potential with distance. The problem of small cells is perhaps best exemplified by the typically low and variable resting potentials recorded upon impalement. Values for resting potential varied greatly from less than 5 mv to more than 40 mv, but the majority of values lay between 5 and 25 mv. Since any cell damage would presumably lower the resting potential, the occasional value greater than 40 mv was probably closer to the true value. Consistent with this interpretation was finding the highest resting potentials in penetrations of the largest cells, e.g., extraembryonic ectoderm. Two points indicate that the damage reflected in the low resting potentials had little effect on the observed coupling: there was no obvious correlation between resting potential and coupling, and coupled pairs of cells were usually separated by many other cells which must also have been coupled but were not impaled.

The blastodiscs were fixed overnight at 0°C in a modified Sandborn (22) fixative containing 6.5% glutaraldehyde and 2% acrolein. The pH was kept at 4.0 with 50% 0.1 M acetate buffer to retain the

TABLE I

| Coupling | No. of pairs of coupled cells |
|--------------------------------------|-------------------------------|
| Within the same tissue | 10 (Figs. 1, 2) |
| Notochord | |
| Paraxial dorsal mesoderm | 2 |
| Somite | 1 |
| Primitive streak ectoderm (epiblast) | —* |
| Neural plate | 3 |
| Neural tube | 1 |
| Lens placode | —* |
| Lens vesicle | 1 (Figs. 3, 4) |
| Limb bud ectoderm | —* |
| Extraembryonic ectoderm | —* |
| Hensen's node | 1 (Fig. 5) |
| Between different tissues | |
| Notochord to neural plate | 7 |
| Notochord to neural tube | 1 (Figs. 6, 7) |
| Notochord to mesoderm | 6 (Fig. 8) |
| Notochord to ectoderm | 1 |
| Neural tube to ectoderm | 1 |
| Hensen's node to mesoderm | 1 |

* Numerous examples of each, but not all filmed; electrode position could be determined without dye marks.

stains during fixation. After dehydration in ethanol, the embryos were cleared in propylene oxide and flat imbedded in Epon. Serial $5\text{-}\mu$ sections were cut with a steel knife.¹

RESULTS

Coupling Between Cells in the Same Tissue

A typical experiment is illustrated in Figs. 1 and 2. Two cells in the developing notochord of an 11 somite embryo were each impaled with a dye-filled

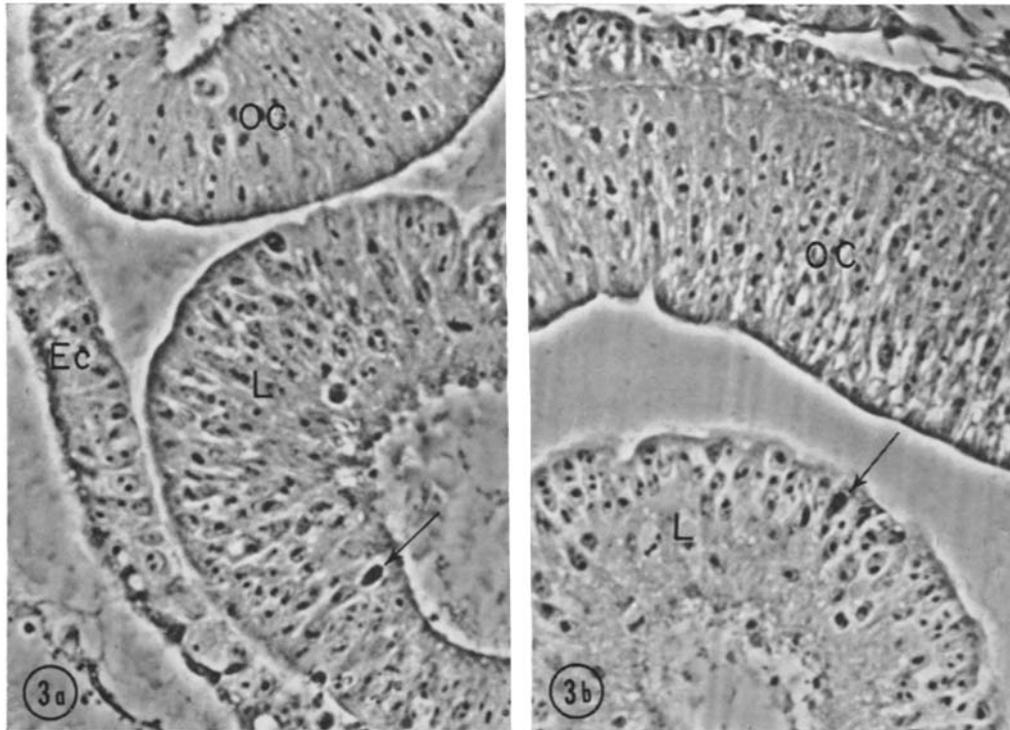


FIGURE 3 *a* and *b* are cross-sections, $85\ \mu$ apart, through the developing lens vesicle and optic cup of an embryo of 3 days of incubation. Arrows indicate two dye-marked cells in the lens vesicle. *L*, lens vesicle; *OC*, optic cup; *Ec*, ectoderm. Phase-contrast. $\times 550$.

micropipette. As in the majority of experiments, penetration was achieved by passing pulses of dye through each micropipette as it pressed against the cell membrane. A negative resting potential immediately appeared upon entry of the cell, and, with a few additional current pulses, the cell could be seen to fill with dye. The current pulse (Fig. 2 *a*) supplied to one cell produced in the second cell an electrotonic potential change (Fig. 2 *c*) of which only a small fraction could be recorded extracellularly (Fig. 2 *b*). The marked cells are shown in Fig. 1.

The extracellular potential change (Fig. 2 *b*) was much smaller than that inside the second cell and essentially the same as that in the bathing solution. Evidently, then, the potential change recorded in the second cell was not the result either of a high-resistance barrier surrounding the whole embryo or of a high interstitial resistance (see references 12, 13, and 16 which deal with similar situations).

In a large number of other cases, electrical

coupling was demonstrated between two cells, but only one cell was adequately marked. These results are of interest only in experiments on ectoderm when the unmarked electrode positions were unequivocal (see Table I). It may be noted that observations during the experiments were often misleading. On a number of occasions a stained cell, which during the experiment or in the fixed and embedded whole mount appeared to be in one tissue, was found in an adjacent tissue when the sections were studied.

In addition to 10 cases of coupling between notochord cells, two cases of coupling between paraxial dorsal mesoderm cells and one case of coupling between two somite cells were found.

Coupling was not limited to mesodermal derivatives, but it was also demonstrated between cells within the ectoderm and its derivatives. Cases of coupling were found in the ectoderm (epiblast) of primitive streak embryos, in the lens placode, neural plate, and neural tube of 10–20 somite embryos, in the lens vesicle and limb

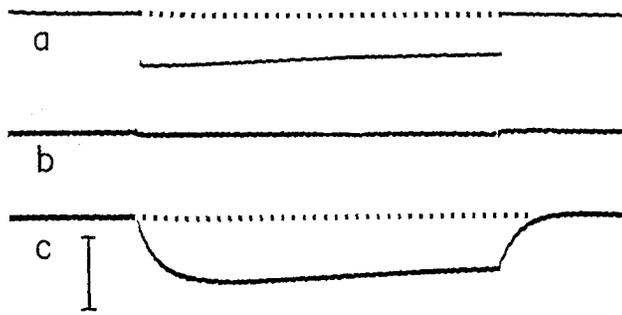


FIGURE 4 Electrical records for cells in Fig. 3. Current pulse in one lens cell (a , 3.5×10^{-8} A for 0.95 sec) produced electrotonic potential change inside (c), but not just outside (b) the second lens cell. Resting potential of second lens cell, 11 mv. Voltage calibration for b and c , 10 mv.

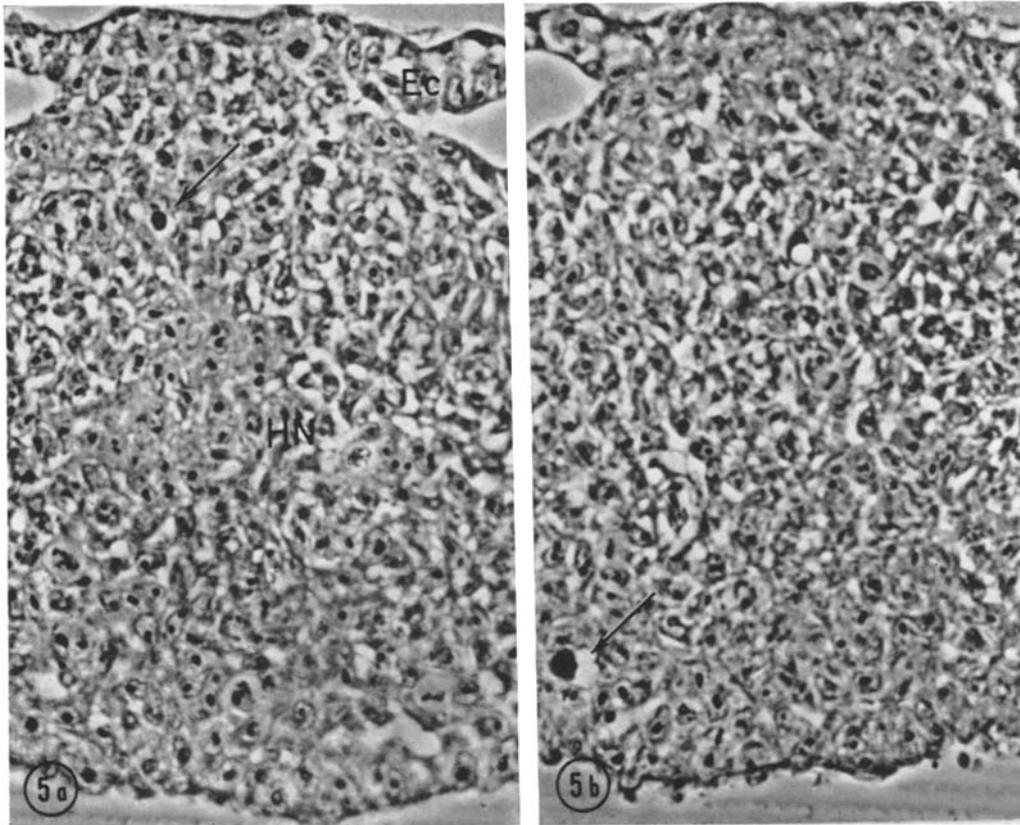


FIGURE 5 a and b are sections, 35μ apart, through Hensen's node in about 19 somite embryo. Arrows indicate two dye-marked cells found to be coupled. *HN*, Hensen's node; *Ec*, ectoderm. Phase-contrast. $\times 550$.

bud ectoderm of 3-4-day embryos, and in extra-embryonic ectoderm of various stages. An example of experiments on lens vesicle is provided in Figs. 3 and 4. Coupling was found in one instance between two cells in Hensen's node (Fig. 5) which had not yet been segregated into neural plate, notochord, and paraxial dorsal mesoderm.

There were no cases where two marked cells in the same tissue were uncoupled, provided they were less than 200μ apart and had a definite resting potential (greater than 5 mv). This result is consistent with the finding that cells are coupled over long distances and implies coupling between intervening cells (see Discussion). However, the

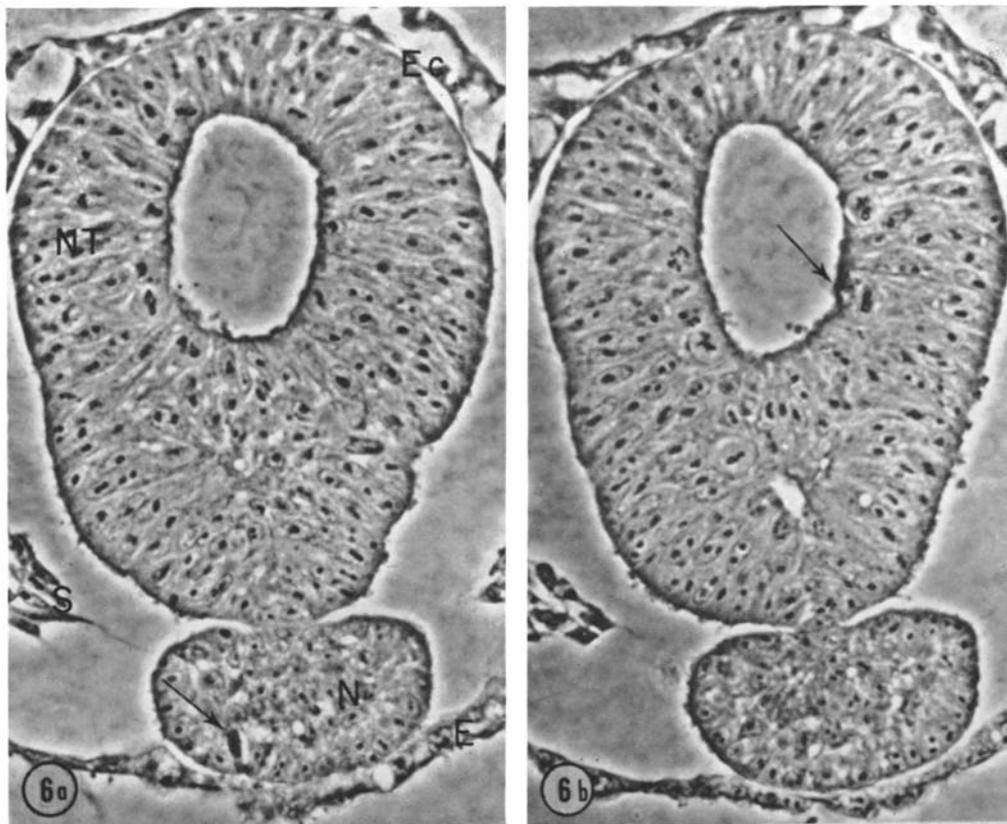


FIGURE 6 *a* and *b* are cross-sections through 16 somite embryo. Arrows indicate dye-marked neural tube cell (*a*) and notochord cell (*b*), $105\ \mu$ apart. *NT*, neural tube; *Ec*, ectoderm; *S*, somite; *N*, notochord; *E*, endoderm. Phase-contrast. $\times 550$.

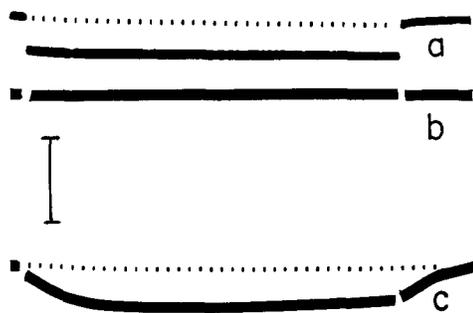


FIGURE 7 Electrical records for Fig. 6. Current pulse in neural tube cell (*a*, 2×10^{-8} A for 0.98 sec) produced electrotonic potential change inside, (*c*), but not just outside, (*b*), notochord cell. Resting potential of notochord cell, 20 mv. Voltage calibration for *b* and *c*, 10 mv.

small number of penetrations in some tissues (Table I) makes it impossible to say whether all adjacent cells are coupled.

Coupling Between Cells in Different Tissues

In addition to coupling between cells in the same tissue, coupling was demonstrated between cells of different developmental potential in the region of Hensen's node. Seven cases of coupling between a neural plate cell and a developing notochord cell were found (23). In one additional case (Figs. 6 and 7), coupling was demonstrated between a notochord cell and a neural tube cell at a stage when the neural tube was complete over most of its length (16 somites). (The neural tube cell was clearly marked with a blue stain, although the contrast does not show up in the phase photomicrograph.)

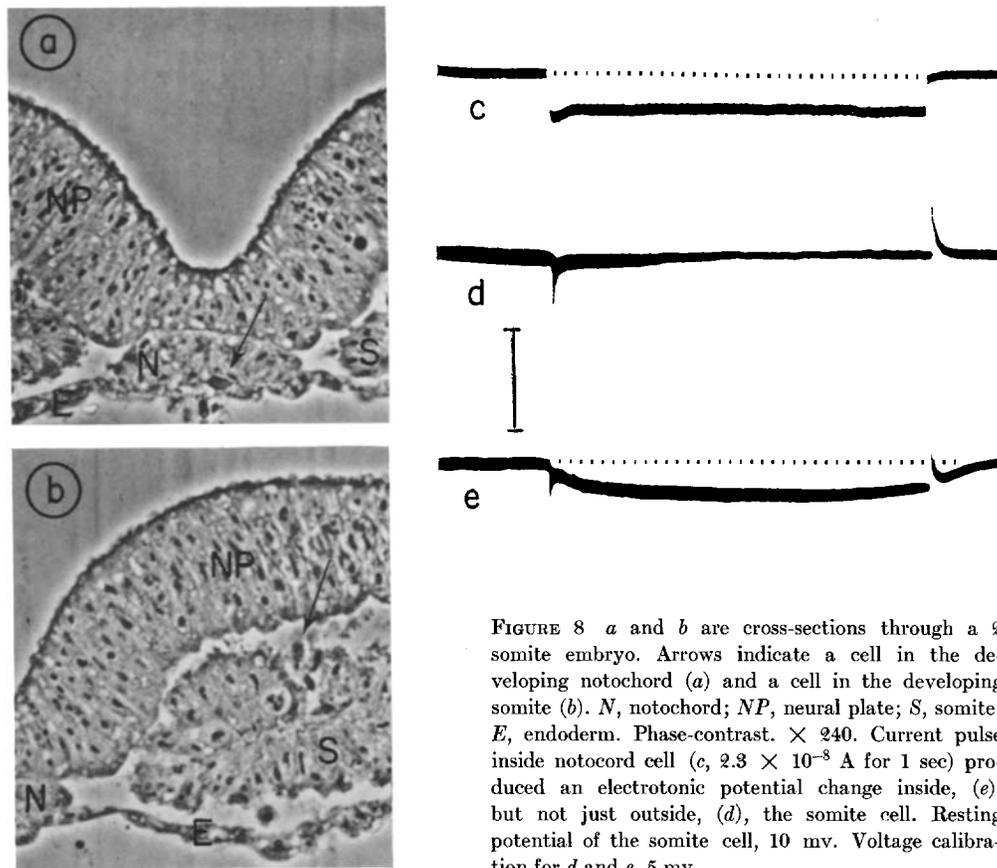


FIGURE 8 *a* and *b* are cross-sections through a 2-somite embryo. Arrows indicate a cell in the developing notochord (*a*) and a cell in the developing somite (*b*). *N*, notochord; *NP*, neural plate; *S*, somite; *E*, endoderm. Phase-contrast. $\times 240$. Current pulse inside notochord cell (*c*, 2.3×10^{-8} A for 1 sec) produced an electrotonic potential change inside, (*e*), but not just outside, (*d*), the somite cell. Resting potential of the somite cell, 10 mv. Voltage calibration for *d* and *e*, 5 mv.

In six cases, coupling was also found between developing notochord and paraxial dorsal mesoderm cells and, in one case, between a notochord cell and an early somite cell (Fig. 8).

Additional cases of coupling between different tissues in the region of Hensen's node are listed in Table I.

An attempt was made to test for coupling between the lens placode or developing lens vesicle and the optic vesicle in 10–20 somite embryos. No coupling could be found. However, the technical difficulties (see Materials and Methods) were greater for these tissues and, therefore, these experiments are not conclusive.

Experiments on Intact Eggs

The question might be asked whether coupling could still be shown with the blastodisc attached to the yolk. This experimental arrangement was not used routinely owing to technical problems with lighting and immobilization of the floating

egg. However, in a few experiments coupling, presumably between surface cells, was demonstrated with 3 M KCl-filled electrodes.

DISCUSSION

The results of the present study indicate that electrical coupling is widespread in the early chick embryo (see Table I). Cells of the same type are coupled in a variety of tissues originating from ectoderm, e.g. neural plate, lens ectoderm, extra-embryonic ectoderm, presumptive epithelium, and from mesoderm, e.g. dorsal paraxial mesoderm, somite, notochord. Electrical coupling also occurs, in the region of Hensen's node, between cells which have different developmental potential and possibly interact (20, 24, 29); i.e. presumptive notochord cells were coupled both to neural plate and to paraxial mesoderm cells.

In most experiments coupling was demonstrated between cells many cell diameters apart. Since electron microscopic studies of the early

chick embryo reveal no cell processes extending over such long distances (27), the ionic current associated with the coupling must have passed through low-resistance channels between the intervening cells. Therefore, each case of coupling in fact implies coupling between many more cells than the two impaled. This fact provides an internal control for the effect of cell damage upon impalement, since the intervening cells are not likely to be directly affected.

In the case of coupling between two cells of the same tissue, the pathway for ion movement involves primarily cells of the same type. However, the current pathway in the cases of coupling between two different tissues requires more comment. Light and electron microscopical studies (24, 27) show that the notochord, neural plate, and paraxial dorsal mesoderm originate at Hensen's node and remain in direct contact via cellular processes for some distance anterior to their origin. Thus the current flowing from notochord to neural plate or mesoderm may pass directly between the two tissues, indirectly through Hensen's node, or via both pathways. The electrical evidence does not distinguish between these alternatives, but the ultrastructural observations discussed below suggest that both pathways are involved.

In their recent electron microscopical study of the early chick embryo, Trelstad et al. (27) have described small areas of intercellular contact where the extracellular space between neighboring cells is apparently obliterated. These "focal tight junctions" seem to be smaller versions of the "tight junctions" (4, 5, 10, 17) found in numerous electrically coupled adult tissues (2, 13), and thus they might be the sites of low resistance between embryonic chick cells. These junctions occur not only between cells within the neural plate, presumptive notochord, and dorsal paraxial mesoderm, but also between presumptive notochord cells and cells of the neural plate and mesoderm; this suggests indirect as well as direct low-resistance channels. Of special interest is the finding that primary mesenchyme cells are connected to hypoblast as well as epiblast cells by focal tight junctions which in these cases are secondary attachments established subsequent to cell migration. However, coupling was not tested between these tissues.

One important unanswered question concerns the pattern of uncoupling during later stages of development. Neural plate and neural tube cells

found to be coupled at early stages are destined to give rise to neural and glial elements. In the adult, glial cells are likely to remain coupled, whereas most neurons will probably lose their coupling with each other and with glial cells, as in the leech and in the mud puppy (11, 12). In addition, some coupled mesoderm and somite cells may give rise to skeletal muscle cells which are not coupled to each other, as well as to smooth muscle and certain connective tissue cells which may be coupled in the adult (3, 18).² Thus the relationship between the time of uncoupling and the differentiation of the neural and somitic tissues would be interesting to learn.

Although microelectrode techniques have been useful in demonstrating spread of ions between cells, they have provided limited information about the function of the low-resistance connections. Where such connections occur between excitable cells, as in the adult heart, they function at least in the transmission of electrical signals (31). However, in numerous other cases, as in the chick embryo, electrical coupling is present between nonexcitable cells, those which do not generate the rapid electrical signals used by nerve and muscle. In these cases, additional functions must be sought for the low-resistance connections.

These connections clearly permit cells to share the pumping or distributing of small ions (16), functions consistent with the widespread occurrence of electrical coupling in epithelia which are often quite active in pumping ions. Another possibility is that the coupled cells can control each other's activity by exchanging larger molecules across the junctions used by small ions (13, 16, 26). Molecules of molecular weights ranging from 305 to 69,000 move freely between electrically coupled cells in insect salivary gland (9, 13). Potter et al. occasionally observed movement of Niagara sky blue: 6 B (molecular weight, about 990) between cells in the squid embryo (16); and spread of this dye occurred sometimes between chick embryo cells, but the inconsistent movement and the strong binding properties of this dye make it a poor tracer. Fluoresceinate ion (molecular weight, 330) has been observed to move freely between electrically coupled mammalian cells in culture³ and between coupled fat cells in the newt

² Potter, D. D., and E. J. Furshpan. Data in preparation.

³ Furshpan, E. J. Personal communication.

(unpublished observations). In no case, however, has it yet been shown that larger molecules move by the same route as the ions. While the mechanisms for the rapid movement of dye molecules between cells are not yet clear, the possibility of direct exchange of molecules of this size between cytoplasm obviously must be kept in mind in considering induction and morphogenetic movements. It is interesting in this regard that electrical coupling is prevalent in the region of Hensen's

node, which is one focal point for the two processes in early chick development (24, 29).

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