ELECTRICAL COUPLING BETWEEN EMBRYONIC CELLS BY WAY OF EXTRACELLULAR SPACE AND SPECIALIZED JUNCTIONS

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ABSTRACT

The meroblastic egg of the teleost, *Fundulus heteroclitus*, was studied electrophysiologically from cleavage to mid-gastrula stages. The yolk is an intracellular inclusion surrounded by a membrane of high resistivity (50 kΩcm²). This membrane generates a cytoplasm-negative resting potential in later stages. Cells of all stages studied are coupled electrically. In gastrulae, coupling is both by way of specialized junctions between cells and by way of intra-embryonic extracellular space, the segmentation cavity. The latter mode is present because the segmentation cavity is sealed off from the exterior by a high resistance barrier, and the outer membrane of surface cells is of high resistance (50–100 kΩcm²) compared to the inner membrane. It can be inferred that clefts between surface cells are occluded by circumferential junctions. Isolated cells from late cleavage stages develop coupling in vitro, confirming the existence of coupling by way of intercellular junctions. Both modes of coupling could mediate communication between cells that is important in embryonic development.

INTRODUCTION

Electrical coupling of cells has been described in embryos of the squid *Loligo* (36), the newt *Triturus* (19, 20), the clawed toad *Xenopus* (42), and the chick (41). Electrical coupling may be of considerable importance in development, for it is possible that the electrical pathways transmit substances between cells that act in the coordination of growth and differentiation.

In the chick embryo, cells of the kinds that were electrically coupled were found to be joined by close membrane appositions, where extracellular space is greatly narrowed or occluded altogether (44, 45). It was proposed that these junctions provide the low resistance pathways between cells. In the squid, embryonic cells are joined by similar close appositions, septate desmosomes, and also cytoplasmic bridges (J. M. Arnold, personal communication). From comparison with other tissues, both types of junction might contribute to the coupling (9) as would the cytoplasmic bridges[1]. Ultrastructural data are lacking for the newt and clawed toad.

[1] Because cell membranes in salivary glands of *Drosophila flavorepleta* have been found to be connected by close appositions, perhaps "gap junctions" (T. Reese, personal communication), there is now reason to question whether septate desmosomes provide low resistance channels between cells.

Recently two types of close membrane apposition have been distinguished, the "gap junction" and the "tight junction", both of which previously had been commonly called tight junctions (12, 40). At the gap junction the membranes appear separated by about 20 A in sections perpendicular to the membranes. This space can be penetrated by the marker substances for extracellular space, lanthanum hydroxide and horseradish peroxidase. In tangential sections the space can be seen to comprise a hexagonal network, and striations at about 90 A periodicity may appear in angled sections. Gap junctions are found as small patches or maculae. They have been described at a number of known sites of electrotonic coupling between cells (12, 40), and the occurrence of 90 A striations suggests that close appositions between other coupled cells are also gap junctions (cf. 9). This association leads to the inference that gap junctions provide low resistance pathways between cytoplasms of the joined cells. It has now been observed that a dye, Procion Yellow M4RS, can pass between cytoplasms through gap junctions connecting segments of the crayfish septate axon and that the dye does not enter the cells from the outside (35). Furthermore, normalized with respect to electrical resistivity the septum is more permeable for intercytoplasmic passage of sucrose than the nonjunctional surface is permeable for efflux of sucrose from the axon (M. V. L. Bennett and P. B. Dunham, Sucrose permeability of junctional membrane at an electrotonic synapse. *Biophys. J.* In press). It can be concluded that permeability of junctional membrane differs qualitatively from that of nonjunctional membrane, and that the gap junctions contain channels between cell cytoplasms that are separated from extracellular space. These channels are most likely to be in the centers of the hexagons seen in tangential sections.

At the tight junction the membranes are seen in perpendicular sections to occlude completely the extracellular space. These junctions occur primarily in epithelia, where they form zonules surrounding the cells (*zonulae occludentes*) that seal off the intercellular clefts and block extracellular passage of substances between the two sides of the epithelia (15). Although zonular tight junctions were formerly considered to couple cells electrotonically, the evidence needs reexamination in light of the distinction between gap junctions and tight junctions. It must be admitted that there is as yet no unambiguous evidence that tight junctions do in fact form low resistance channels between cell cytoplasms. The uncertainty remains because no electrotonically coupled cells have been shown to be joined exclusively by tight junctions, and because at least some coupled epithelial cells are joined by both gap and tight junctions (40).

Since there is considerable knowledge of early teleost development (14, 33), especially in terms of cell contacts, cell movements, and fine structure (26, 47, 51), it seemed desirable to extend the electrical studies to teleost eggs. This paper reports findings on embryos of the killifish *Fundulus heteroclitus* that have some general relevance to relations between cells and properties of intracellular membranes, in this instance, the membrane surrounding the yolk.

The egg of *Fundulus* develops meroblastically (1), like all teleost eggs. Prior to fertilization, the large, spherical, fluid yolk mass is contained within a single membrane-bounded sac that is enclosed in a layer of cytoplasm with an external limiting membrane on its surface. Following egg activation, most of the cytoplasm streams to the animal pole where it forms a cap, the blastodisc. A thin layer of cytoplasm (about 5 μ thick) covers the yolk over the remainder of the egg. This layer has been termed the yolk cytoplasmic layer (26, 51).

Cleavage is confined to the blastodisc and does not involve the yolk. The earliest cleavage divisions remain incomplete at the lower surface of the blastodisc and around its edges. These cell boundaries are completed with later cleavages, but a layer of uncleaved cytoplasm containing many nuclei is left between the cellular blastoderm and the yolk. This multinucleate layer or periblast is continuous with the nonnucleated yolk cytoplasmic layer which encloses the rest of the yolk (Fig. 1). The yolk is separated from the periblast and yolk cytoplasmic layer by the yolk membrane. Thus, the yolk is actually contained within a huge vacuole of a multinucleated "yolk cell" consisting of the periblast and yolk cytoplasmic layer. The yolk membrane is highly convoluted beneath the periblast, suggesting specialization of function in this region. The cellular blastoderm undergoes cleavage into several thousand cells and then flattens and spreads to surround the yolk cell in an extensive movement termed epiboly. By this process the

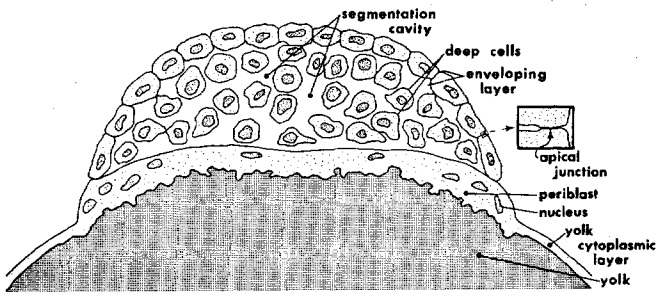

FIGURE 1 Diagram of an early blastula (stage 8). The lower part of the egg has been omitted. The *inset* shows the apical region between two cells of the enveloping layer. Modified from (26).

yolk cell becomes entirely enclosed in multiple layers of cells out of which the embryo develops. Throughout early development all surface cells are tightly adherent to each other. Because this surface layer covers the entire blastoderm (Fig. 1) and envelops the whole egg after closure of the "blastopore" or yolk plug, it has been termed the enveloping layer.

During all stages from cleavage through epiboly, the surface cells are joined to each other along their entire apical margins by circumferential junctions, in which their plasma membranes are in close apposition in a continuous band around the cells. Over most of the apposition, the plasma membranes are separated by a distance varying from approximately 20 to as much as 75 A. At the surface and at irregular intervals along the apposition, however, the membranes are seen in uranyl acetate-stained material (23) to come together to form tight junctions where the extracellular space is completely occluded (Lentz and Trinkaus, unpublished data, and Fig. 14–10 in reference 49). This association of regions of close apposition with tight junctions is probably typical of circumferential junctions (cf. 12). These regions of close apposition lack the hexagonal structure characteristic of gap junctions and may only serve an attachment function. The physiological evidence indicates that the tight junctions form *zonulae occludentes*, although continuity around the cells' apical margins has not been demonstrated morphologically. Below this apical junction the plasma membranes diverge to produce an irregular intercellular cleft, usually about 200 A across, but that in places expands to form large spaces. What are apparently gap junctions are found in this deeper region in gastrulae, but they have not yet been seen in earlier stages. At these junctions the membranes of adjacent cells converge and approach each other up to a distance of 20–30 A, but do not fuse (Lentz and Trinkaus, unpublished data).

Between the enveloping layer and the underlying periblast are the so-called deep cells (Fig. 1), a relatively loosely packed population of cells that show intense surface activity and extensive translocation during late blastula and gastrula stages. They are less closely joined structurally than cells of the enveloping layer. Occasionally, they make what may be small gap junctions with other deep cells and the undersurface of enveloping layer cells, but mostly they are separated by 200 A or much wider spaces. The many large spaces between deep cells constitute the segmentation cavity of the blastoderm. This cavity is filled with extracellular fluid which probably contains nutrient products transmitted from the yolk through the periblast.

The electrical properties defined by the present study can be readily summarized. The yolk is an intracellular inclusion, surrounded by a high resistance membrane that separates it from the cytoplasm of the yolk cell, i.e., the periblast and yolk cytoplasmic layer. The interiors of all cells and the segmentation cavity are closely coupled electrically. In many respects these regions can be considered to comprise a single electrical compartment. We have termed this the embryonic compartment. At least in respect to surface cells, current spreads between them by way of both the extracellular space of the segmentation cavity and intercellular junctions. It is possible for current to spread by way of the segmentation cavity because the outer membrane of cells of the enveloping layer is of very high resistance, and at least part of the membrane abutting on the segmentation cavity is of relatively low resistance. Furthermore, there is an effective barrier between the segmentation cavity and the exterior that is presumably provided by the apical tight junctions between the cells of the enveloping layer.

Part of this work has been reported previously (10, 11).

METHODS

Eggs of *Fundulus heteroclitus* were procured from various locales near Woods Hole, Massachusetts, and fertilized by standard procedures outlined by Trinkaus (48). The stages of development were those defined by Oppenheimer (32). The eggs were prepared for electrophysiological study by mechanically removing the tough chorion with watchmakers' forceps or iridectomy scissors. Extreme care was exerted to avoid visibly injuring the eggs in any way. During the electrical studies, the eggs were immersed in unbuffered double strength Holtfreter's solution in small petri dishes (120 mM NaCl, 1.3 mM KCl, 0.5 mM $CaCl_2$). This solution was used because, even though *Fundulus* eggs develop in sea water and in all dilutions of it down to distilled water, they submit to operative procedures better and close wounds more readily in double strength Holtfreter's solution than in all other solutions tested (50). Eggs were usually dechorionated at the single-celled blastodisc stage or two-cell stage.

Conventional electrophysiological techniques were employed (9). Separate glass micropipettes, filled with 3 M KCl and of 10–20 MΩ resistance, were used for recording and for passing current. Voltages were recorded with respect to an Ag-AgCl indifferent electrode in the bath. A separate Ag-AgCl bath electrode was used to complete the circuit for current pulses. Electrodes were easily pushed into the yolk, although often there was a large indentation of the surface before an electrode suddenly penetrated. Penetration was often aided by passing cathodal current pulses. If any indentation remained following penetration, it was relieved by retracting the electrode a suitable distance. The yolk cytoplasmic layer has the ability to close large holes in itself within a few minutes (46). Thus, if a small tear was made inadvertently during penetration, this layer would close the wound, surround even a large diameter electrode, and quite tightly seal off the site of entry into the yolk. Electrodes could be placed in cleavage blastomeres and in cells of an early blastula under visual control, but in gastrula stages, when the cells became quite small, the intracellular location of the electrodes was inferred primarily from the presence or absence of a resting potential.

The electrical equivalent of the egg (Fig. 9) is similar to that used for electrotonic junctions (6), and formulae for calculating the resistances of its components from measured input and transfer resistances are given in that paper. (The input resistance in a given compartment is the voltage in that compartment divided by the current applied in that compartment. The transfer resistance from one compartment to a second compartment is the voltage in the second compartment divided by the current in the first compartment.) In most experiments, transfer resistances in the two directions (from yolk to embryonic compartment and vice versa) were equal to within 10%. Absolute equality should in principle be found in a linear system. Where transfer resistances differed by more than 10%, the data were rejected, although it usually could be inferred that the error was due to faulty placement of the current electrode in the cytoplasm of a surface cell. The average of transfer resistances in the two directions was used for most calculations.

When the leakage resistance R_l was large (Fig. 9), a small error in measurement of transfer resistance and of input resistance in the cytoplasm could introduce a large error in R_l. In these cases, R_l was estimated from the transfer resistance from cytoplasm to yolk, r_{cy}, the input resistance in the cytoplasm, r_{ee}, and the resistance of the yolk membrane calculated in the usual way. These resistances are related as follows:

$$R_l = \frac{R_{ym}\, r_{cy}}{r_{cc} - r_{cy}}$$

Except where otherwise indicated each statement in the results is based on three or more experiments.

RESULTS

The characterization of the different membranes in the egg required that current and recording electrodes be placed in a relatively large number of positions. Usually a current and a voltage electrode were inserted into the yolk, and then three other electrodes were placed in the sites relevant for that particular experiment.

When rectangular current pulses were applied, potentials were generated that reached steady-state values rather slowly, i.e., over periods of 0.1 sec or more. The slowness of the changes indicates that the membrane time constants are quite long, as will be considered below. The steady-state voltages produced by inward (cathodal or hyperpolarizing) pulses increased linearly with current strength over a moderate range. This finding demonstrates that the membranes behave like fixed resistances under these conditions. In view of this fact, most calculations of resistance were based on a single value of inward current. In early cleavage stages outward currents appeared to produce a small increase in conductance (rectification). The change was probably in the surface membrane, but it was not investigated sufficiently to warrant further discussion.

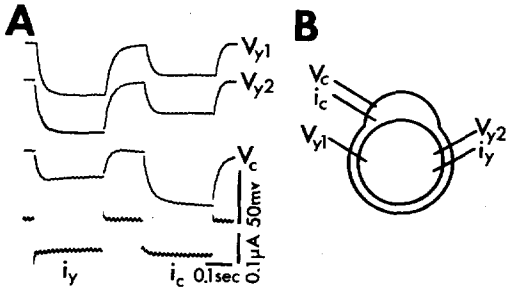

FIGURE 2 Isopotentiality of the yolk. Data from a 128-cell stage. *A*. Oscilloscope record. *B*. Diagram showing electrode placements (cf. Figs. 1, 9). The upper three traces record voltages generated by the currents shown on the lowest trace. Positive voltages and outward currents are indicated by upward deflections in this and subsequent figures. As indicated in *B*, the two recording electrodes in the yolk (V_{y1} and V_{y2}, upper two traces) were at opposite sides of the egg, and the current electrode in the yolk (i_y, first pulse) was close to one of them. Voltage and current electrodes (V_c, third trace; i_c, second pulse) were also placed in a surface cell. The potentials recorded by the yolk electrodes were identical, but differed from the potential in the surface cell.

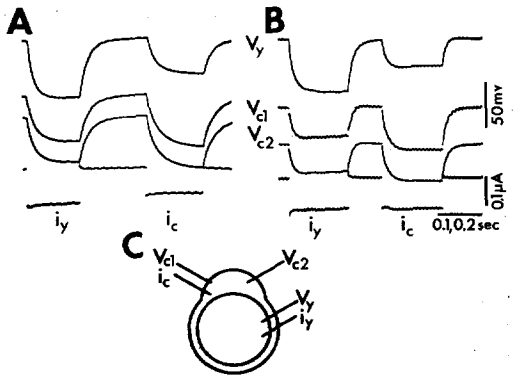

FIGURE 3 Isopotentiality of cytoplasm in cleavage stages. *A*, *B*. Oscilloscope records from a four-cell stage and from a 128-cell stage. Voltages on upper three traces; currents on lowest trace. *C*. Diagram of electrode placements. For *A*, recording electrodes were placed in adjacent blastomeres (V_{c1} and V_{c2}, second and third traces) and a current electrode (i_c, second pulse) was placed in the one from which V_{c1} was recorded. For *B*, recording electrodes were placed in widely separated surface cells (V_{c1} and V_{c2}) and again a current electrode was placed in the cell from which V_{c1} was recorded. For both *A* and *B*, there were recording (V_y, upper trace) and current (i_y, first pulse) electrodes in the yolk. The potentials recorded in the two cells were identical (*A*) or nearly so (*B*), but differed from the potential in the yolk.

Isopotentiality of the Yolk

Two electrodes anywhere in the yolk (Fig. 2 *A*, upper two traces) recorded the same potential whether current was applied by a third electrode in the yolk (i_y, first pulse in Fig. 2 *A*) or by an electrode in the embryonic compartment (i_c, second pulse in Fig. 2 *A*). These data indicate that the yolk is isopotential and that it constitutes a single compartment electrically. The potential in the yolk was always greater than that in the embryonic compartment (V_c, third trace) when current was applied in the yolk. The potential in the yolk was usually less than that in the embryonic compartment when current was applied in the embryonic compartment. Thus, there must be a resistive barrier between the yolk and the embryonic compartment. (If the tip of a recording electrode were placed very close to the tip of a current electrode, an additional voltage would be recorded due to voltage drop across the resistance of the yolk or cytoplasm. The amplitude of this component would be inversely proportional to the distance between tips, and calculations indicate that it would be negligible for separations greater than about 10 μ). The isopotentiality of the yolk was tested as in Fig. 2 from early cleavage to gastrula stages.

Electrical Coupling of Cells in Cleavage and Blastula Stages

The cytoplasm in cleavage and early blastula stages is, like the yolk, isopotential, or nearly so. For the records shown in Fig. 3 *A*, adjacent blastomeres of a four-cell stage were each penetrated by recording electrodes (second and third traces). Current was applied through a second electrode in the cell whose potential is shown on the middle trace. A recording electrode (upper trace) and a current electrode were also placed in the yolk. The potential in one blastomere was the same as that in the other, whether current was applied in the yolk (first pulse) or in the cytoplasm of one of the blastomeres (second pulse). The potential in the blastomeres was greater than that in the yolk when current was applied in a blastomere, and smaller than that in the yolk when current was applied in the yolk. Identical results were obtained when diagonally placed blastomeres were studied.

The virtually complete coupling of cells in early stages is hardly surprising, since cleavage is partial

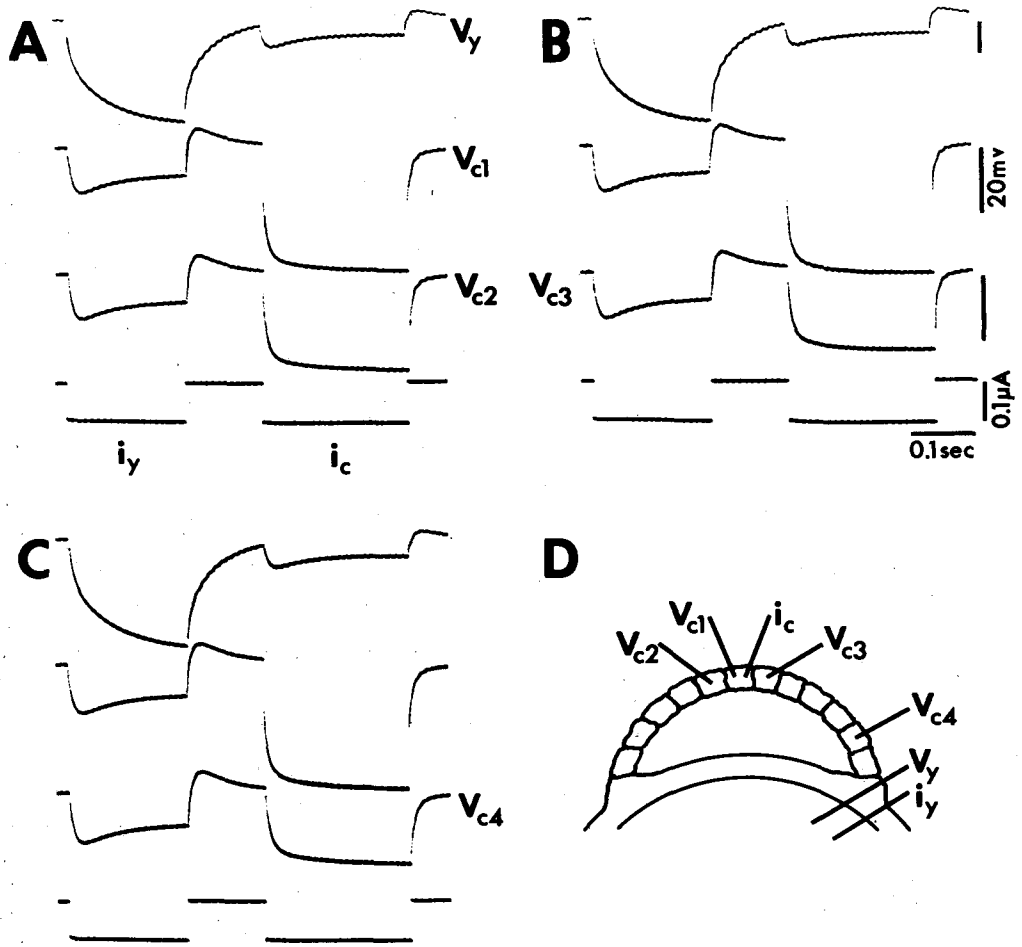

FIGURE 4 Barrier between cells in blastula stages. Data from a blastula at stage 8. *A–C*. Oscilloscope records; voltage on upper three traces, current on lower trace. *D*. Diagram of electrode placement. There were voltage and current electrodes in the yolk (V_y, first trace; i_y, first pulse). There were voltage and current electrodes in a superficial blastomere (V_{c1}, second trace; i_c, second pulse). A fifth exploring electrode recorded voltage (third trace) in each of two adjacent cells (*A*, *B*) and in a distant cell (*C*). The potential due to current applied in the blastomere was largest in the polarized cell. In the adjacent cells it was much smaller, and only slightly greater than in the distant cell. The cells were isopotential when current was applied in the yolk, confirming that the electrodes were intracellular.

and there is cytoplasmic continuity between cells. Close coupling, however, persists into later cleavage stages, where cleavage is complete for the surface blastomeres. (It is still incomplete at these stages for lower blastomeres and the periblast, the region of the yolk cell under the blastoderm.) The data shown in Fig. 3 *B* were taken from an embryo of about 128 cells. The procedure was the same as that for Fig. 3 *A*; two electrodes were placed in a superficial blastomere somewhat removed from the margin of the yolk cytoplasmic layer (second trace and second current pulse). Another recording electrode was placed in a blastomere near the opposite edge of the embryo (third trace). Voltage and current electrodes were also placed in the yolk (first trace and first current pulse). The potential in the two blastomeres was equal when current was applied in the yolk. When current was applied in one blastomere, however, the potential in that cell was 18% greater than the potential in the distant cell (second pulse). Apparently by this stage, the cell membranes provide some barrier

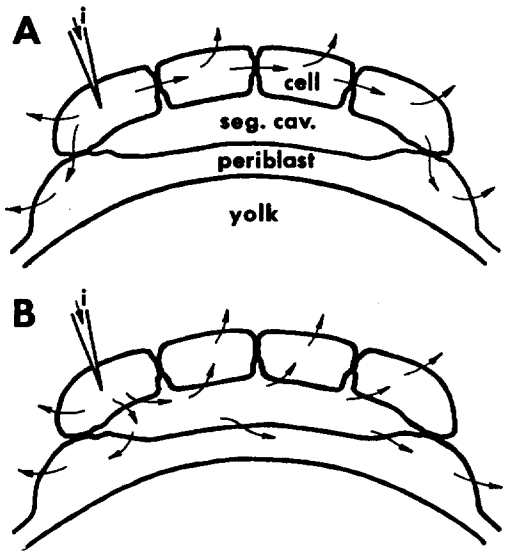

FIGURE 5 Possible electrical pathways coupling cells. A current i is applied by means of an intracellular microelectrode and flows as indicated by the arrows. *A*. Current passes from cell to cell by way of low resistance junctions between them. *B*. Current passes from cell to cell by way of the segmentation cavity, the membrane that faces on the cavity being of low resistance compared to the surface membrane and the cavity being sealed off from the exterior by circumferential junctions.

between cells, but one that is small compared to the surface resistance.

As the cells become smaller, the barriers between them become more significant. Fig. 4 is from a blastula stage (about stage 8) in which the cells were 30–50 μ in diameter. When current was applied in a cell, the potential in it could be almost twice as large as that in a distant cell (*C*). Most of the potential drop occurred between the polarized cell and its immediate neighbors; these cells were at only slightly higher potentials than distant cells (*A*, *B*).

Whereas coupling of cells in early cleavage stages is ascribable to cytoplasmic continuity, there are two other possible pathways by which cells of later stages could be coupled. Current could flow from cell to cell across specialized junctions between them, such as gap junctions, or, less likely, tight junctions (Fig. 5 *A*). Alternatively, circumferential tight junctions could electrically seal off the segmentation cavity, and then, current could proceed from cell interior to cell interior by way of the segmentation cavity, as diagrammed in Fig. 5 *B*. For there to be close coupling by this pathway, the resistance of membranes abutting on the segmentation cavity must be low compared to that of the external surface membranes. This requirement could be easily met, for the surface membranes are of very high resistance compared to most known biological membranes (see below).

The experiments on the gastrula described in the next section show that at least at this stage current can pass from cell to cell by way of both pathways, i.e., across specialized junctions between cells, and through the extracellular space comprising the segmentation cavity.

Coupling in the Gastrula Stage

At this stage of development, the cells are quite small and it was difficult to be sure visually that an electrode was intracellular. However, by this stage the cells have developed internally negative resting potentials (Fig. 10) and penetration of a cell was signalled by a negative shift in the baseline. Continuing to move an electrode inward resulted in a loss of resting potential, and in favorable cases it was clear that the electrode had entered the underlying segmentation cavity.

The segmentation cavity is isopotential, whether a current pulse is applied in the yolk or in the segmentation cavity. This was shown using two recording electrodes in the segmentation cavity in the same manner as illustrated in Fig. 3 *A*. Furthermore, an electrode recorded the same potential in a cell of the enveloping layer, in a deep cell, and in the segmentation cavity when current was applied in the yolk. In addition, these sites were also isopotential when current was applied in the segmentation cavity. In the experiment of Fig. 6, a recording electrode (second trace) was in a deep cell and a current electrode was in the segmentation cavity, as judged by apparent position and respective presence and absence of resting potential. Also, a recording electrode (first trace) and a current electrode (first pulse) were in the yolk. A fifth electrode was advanced into the blastoderm, and just beneath the surface a resting potential was recorded, indicating penetration of a superficial cell (Fig. 6 *A*; the dotted line indicates the outside potential). The potentials due to current pulses that were recorded inside the superficial cell were nearly identical to those in the deep cell, whether current was applied in the yolk (first pulse) or in the segmentation cavity (second pulse). The exploring electrode was then advanced

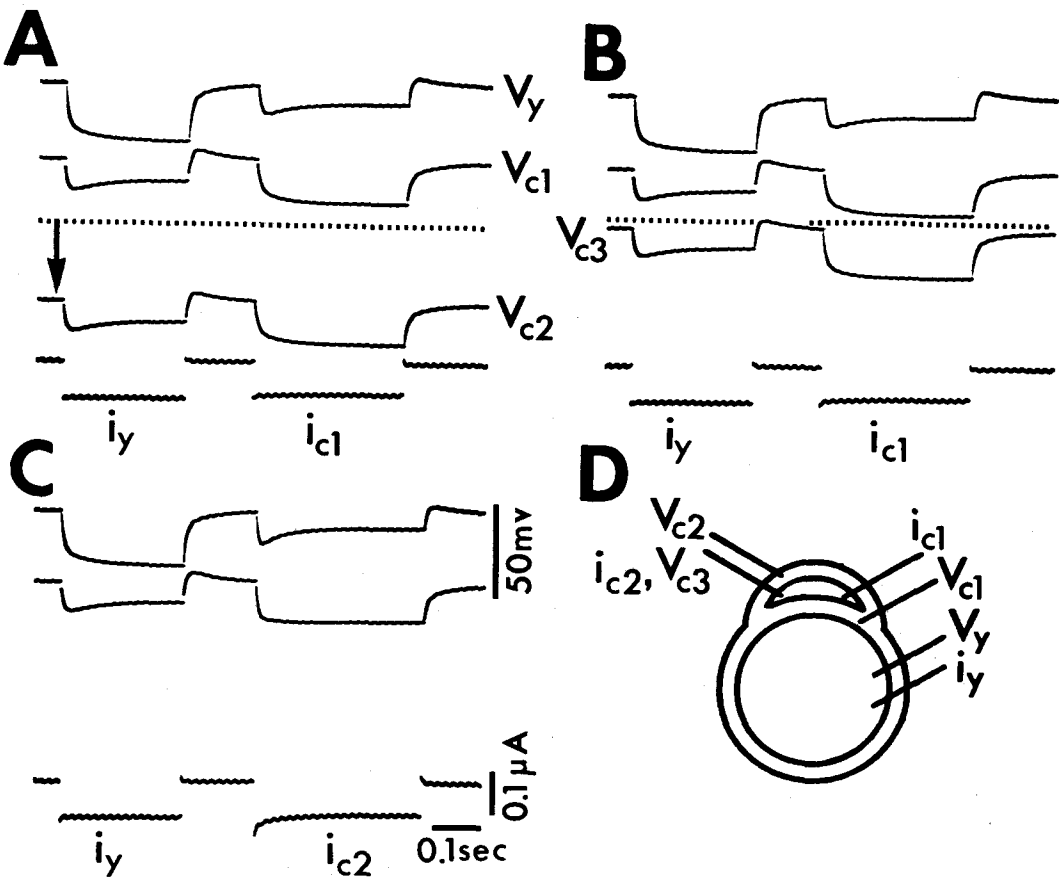

FIGURE 6 Coupling of the cytoplasm and segmentation cavity in the gastrula. *A–C*. Oscilloscope records; voltages on upper three or two traces, currents on lowest trace. *D*. Diagram of electrode placement. For all records there were voltage and current electrodes in the yolk (V_y, upper trace; i_y, first pulse). There were also a voltage electrode in a deep cell (V_{c1}, second trace) and a current electrode nearby in the segmentation cavity (i_{c1}, second pulse in *A* and *B*). *A*. At some distance from the electrode in the deep cell a fifth electrode was advanced into the blastoderm. Close to the surface this electrode recorded a negative resting potential, indicating that a cell of the enveloping layer had been penetrated (V_{c2}, outside potential shown by the dotted line). Potentials generated by the applied currents were also recorded. These potentials were very nearly the same in superficial and deep cells. *B*. When the electrode was advanced farther, there was a loss of resting potential, indicating that the electrode had entered the segmentation cavity. (The dotted line shows the outside potential on removal of the electrode.) The evoked potentials were nearly equal in the segmentation cavity and deep cell. *C*. Current was applied through the exploring electrode when it was in the segmentation cavity (i_{c2}, second pulse). The resulting potentials in the deep cell and yolk were about the same as when a similar pulse was applied through the other electrode in the segmentation cavity. The difference in shape is due to change in resistance of the exploring electrode during the pulse.

farther, and the loss of resting potential indicated that it had penetrated the segmentation cavity (Fig. 6 *B*). As when it was in the superficial cell, it recorded very nearly the same potentials during current pulses as in the deep cell. When current was passed through the exploring electrode, the effect was nearly the same as when current was passed by means of the other electrode in the segmentation cavity (Fig. 6 *C*). There was a small difference in shape due to the differences in the

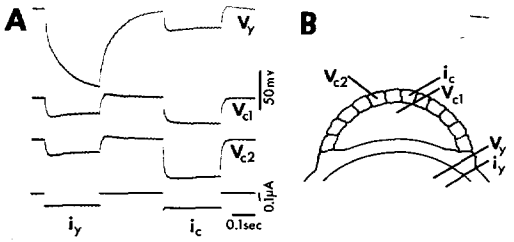

FIGURE 7 Coupling of cells of the enveloping layer by way of specialized junctions. Early gastrula. *A*. Oscilloscope records. *B*. Diagram of electrode placement. Voltage and current electrodes were placed in the yolk (V_y, first trace; i_y, first pulse on lowest trace). A second voltage electrode was placed in the segmentation cavity (V_{c1}, second trace). A third electrode was placed in a cell of the enveloping layer close to the electrode in the segmentation cavity (V_{c2}, third trace). Current was applied in a neighboring superficial cell (i_c, second pulse). Since the cells penetrated by electrodes were clearly separated by at least one intervening cell, the electrodes could not have been in the same cell. The voltage in the superficial cell that was produced by the current in the neighboring cell was about 50% larger than the voltage in the underlying segmentation cavity. The voltage in the superficial cell produced by the current applied in the yolk was slightly smaller than the potential in the segmentation cavity, either because the electrode had not completely penetrated the cell, or because it had caused a small leak to the exterior.

current pulses; the resistance of the exploring electrode increased during passage of current, with the result that the pulse was not rectangular.

These results indicate both that there is little barrier to current flow in the segmentation cavity and that the cells of the enveloping layer are so closely apposed that little current can pass between cells to the outside. The resistance of the inner membrane of the cells of the enveloping layer is clearly low compared to that of the outer membrane, and thus current can flow between cells as diagrammed in Fig. 5 *B*.

That current can pass between cells of the enveloping layer by way of junctions between them is indicated by results like those shown in Fig. 7. In this experiment, one electrode was placed in the segmentation cavity close to where two electrodes penetrated separate, but neighboring cells of the enveloping layer. When current was passed into one cell, a potential was recorded in the neighboring cell that was larger than the simultaneously recorded potential in the underlying segmentation cavity. Since the potential recorded in the cell was greater than that in the underlying segmentation cavity, it can be concluded that current spread from the other cell by means of intercellular junctions as in Fig. 5 *A* and not by way of the segmentation cavity as in Fig. 5 *B*. The potential in cells of the enveloping layer fell off rapidly with distance from an intracellular current electrode and became equal to the potential in the segmentation cavity (cf. Figs. 4, 6). If the current electrode was pushed into the segmentation cavity, there was little change in potential in the segmentation cavity, but the potential in the nearby superficial cell immediately dropped to the same value as in the segmentation cavity. Thus, although a small region of the outer plasma membrane had a higher potential across it when current was applied within a surface cell than when current was applied in the segmentation cavity, the current though this region was small compared to the current through the entire surface.

The foregoing experiments allow no conclusion concerning the mode of coupling of deep cells. These cells may be coupled to each other, superficial cells, or periblast by way of specialized junctions, or they may lie free in the segmentation cavity and be coupled solely by way of extracellular space.

Coupling between Isolated Blastomeres

Additional evidence for coupling by way of junctions between cells was obtained by isolating upper blastomeres from late cleavage stages (in more than 10 experiments at stages of 32 to 256 cells). This procedure should in effect short circuit the segmentation cavity and prevent current flow as in Fig. 5 *B*. Coupling was observed between cells that had been attached in vivo and also between cells that had been separated and then pushed together and allowed to adhere to each other (Fig. 8). The formation of coupling in cells that had been separated required about 1 hr. The degree of coupling varied greatly. It could be so close that one could not exclude cytoplasmic continuity. The time course was not studied, because development of coupling was impeded by microelectrode penetration. The initial contact of cells usually resembled contact between billiard balls. The area of adherence then increased until often the two cells formed a sphere of which each was a hemisphere. Electrical coupling could be very small even though cells adhered over a large area.

The Equivalent Circuit of the Embryo and Membrane Properties

The foregoing results indicate that during very early stages the embryo consists of two compartments electrically, i.e., the yolk is at a nearly constant potential throughout, and the cytoplasm is similarly isopotential. The two compartments are separated by the yolk membrane, which has a significant resistance, since the potential in the yolk can be quite different from that in the cytoplasm. The isopotentiality of the yolk is consistent with the absence of membranous divisions of the yolk. The electrical data also show that in late cleavage stages the resistance of membranes between cells is low compared to the resistance of the pathways to the exterior. Thus, the egg can be represented as having only yolk and surface membranes, and a resistance and capacity can be assigned to each membrane (Fig. 9). There may also be small cytoplasmic compartments that were not penetrated by the electrodes.

The observations require that there is a pathway that directly connects the yolk to the exterior and does not pass through the cytoplasm of the blastomeres. If there were not such a path, current applied in the cytoplasm would produce a potential in the yolk that was equal to, instead of less than, the potential in the cytoplasm. It will be shown below that this direct path is due mostly if not entirely to leakage to the exterior around the electrodes penetrating the yolk. Thus, in early stages the embryo can be represented by the equivalent circuit shown in Fig. 9.

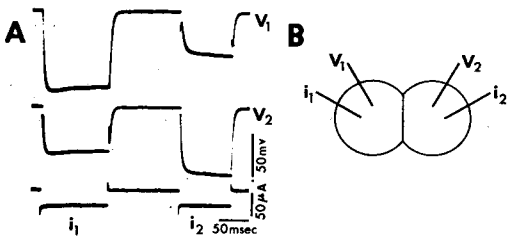

FIGURE 8 Coupling of isolated blastomeres. *A*. First two traces, voltages in two cells (V_1, V_2), each about 100 μ in diameter (from a 128-cell stage). Third trace, current applied through an electrode in each cell (i_1, i_2). *B*. Electrode placements.

As noted in respect to the isopotentiality of the yolk, there is an increased potential close to the tip of a microelectrode that is passing current. This increase is due to the volume resistivity of the yolk or cytoplasm which becomes significant as the current density increases close to a small source of current. In late blastula and gastrula stages the cell membranes become a significant barrier between cells (Figs. 4, 7); a cell in which current is applied and its immediate neighbors can be at higher potentials than distant cells. As distance from the polarized cell increases, the potential rapidly approaches that in distant cells. Thus, the equivalent circuit of Fig. 9 also applies to later stages, provided that potentials in cells close to an intracellular current electrode are excluded.

The electrical measurements and morphological data indicate that in the gastrula the surface membrane corresponds to the membrane of cells of the enveloping layer peripheral to the circumferential junctions that interconnect them. In earlier stages where electrodes were not placed in the segmentation cavity, the membrane that is electrically on the surface could include membrane lining the segmentation cavity, as far as the electrical measurements are concerned. However, the morphological similarities of the intercellular junctions at successive stages suggest that the segmentation cavity of blastulae is also sealed off from the exterior.

From the equivalent circuit and data like those in Figs. 2–4, 6, and 7, resistances of the yolk and surface membranes and of the leakage path were calculated (see Methods). The results of a number of experiments are shown in Table I. Since the surface of the egg is about 0.1 cm² in area (about

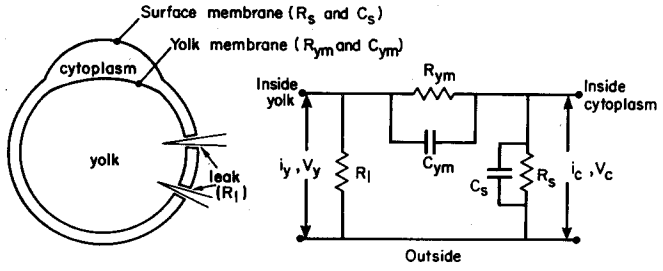

FIGURE 9 Diagram and equivalent circuit of the embryo. V, i, R, and C represent voltage, current, resistance, and capacity, respectively. The subscripts y, ym, l, s, and c represent yolk, yolk membrane, leakage, surface, and cytoplasm, respectively.

TABLE I

Membrane and Leakage Resistances during Development.

Calculations according to the equivalent circuit of Fig. 9. Each group is arranged in order of decreasing R_l. Means are not given for R_l because of its variability.

Stage	R_l	R_{ym}	R_s
		$M\Omega$	
2–4 cell	13.00	0.40	1.30
	5.20	0.45	1.30
	1.80	0.90	1.20
	0.95	0.50	1.20
mean		0.55	1.25
Blastula	16.00	0.90	2.25
	2.20	0.85	1.40
	2.20	0.55	0.10
	1.20	0.80	0.45
	1.20	0.45	0.35
	1.10	0.55	0.65
	0.80	0.65	0.70
mean		0.7	0.85
Gastrula	∞	0.55	0.70
	∞	0.50	0.65
	∞	0.40	0.50
	2.20	0.60	0.75
	1.00	1.50	0.25
	0.35	0.50	0.75
	0.40	0.45	0.30
	0.35	0.60	0.75
mean		0.65	0.60

1.8 mm diameter), the specific membrane resistivities of the yolk and surface membranes are about 50–100 $k\Omega cm^2$. A few measurements indicated that the high resistance of the yolk and surface membranes persisted at least as late in development as the onset of circulation (stage 22). (Somewhat lower resistances than observed here were measured between yolk and exterior by Kao [21]. In his experiments electrodes were pushed into the yolk through the very tough chorion and the lower resistances obtained were probably a result of injury.)

The membrane resistance of isolated blastomeres ranged from several hundred to several thousand Ωcm^2 (about 1 $k\Omega cm^2$ for the cells in Fig. 8). These resistances are about 1% or less of the surface and yolk membrane resistances and may correspond to the resistance of membrane abutting on the segmentation cavity.

The time constants of yolk and surface membranes were difficult to evaluate accurately, because the potentials recorded were not exponential, when R_l was appreciable and the time constants of yolk and surface membranes differed (Fig. 9). In the experiments where R_l was quite large, the potentials in the cytoplasm and across the yolk membrane did change approximately exponentially. In these experiments, the time constants were about 40–50 msec. The time constants of isolated blastomeres were much shorter, corresponding to their lower surface resistances. Insufficient data were obtained for reliable calculation of specific membrane capacitance, but it appeared to be somewhat less than 1 $\mu F/cm^2$.

When R_l was low and current was applied in the cytoplasm, the potential in the yolk rose to a maximum and then fell back, while the potential in the cytoplasm rose monotonically to the steady-state value (Figs. 6 and 12). At the end of the pulse, the potential "overshot" the resting value and then returned to it. This form of potential change was obtained because the yolk membrane had a time constant greater than that of the leakage path (6). The leakage path is presumably a pure resistance and therefore has a negligibly small time constant. Often, a similar result was obtained when pulses were applied in the yolk. In these cases, current applied in the yolk generated a potential in the cytoplasm that rose to a maximum and then decreased, while the potential in the yolk increased monotonically. The converse changes followed the end of the pulse (Figs. 2, 6, 7). The pathway across the surface membrane to the exterior must have had a lower time constant than did the yolk membrane, perhaps as a result of leakage around the electrodes penetrating the cells.

In early cleavage stages, the resting potentials in both yolk and cells were quite small, less than 20 mv cytoplasm side negative (Fig. 10). During blastula and gastrula stages, the resting potentials in cytoplasm (including that of the yolk to cytoplasmic layer, Fig. 13 *C*) progressively increased to about 50 mv inside negative. In the segmentation cavity of the gastrula, little resting potential was recorded, which indicates that the external and the internal surfaces of the cells generated more or less equal potentials. At all stages, little potential was recorded between the yolk and outside. Since the potential in the yolk is the difference between the potentials generated by the yolk

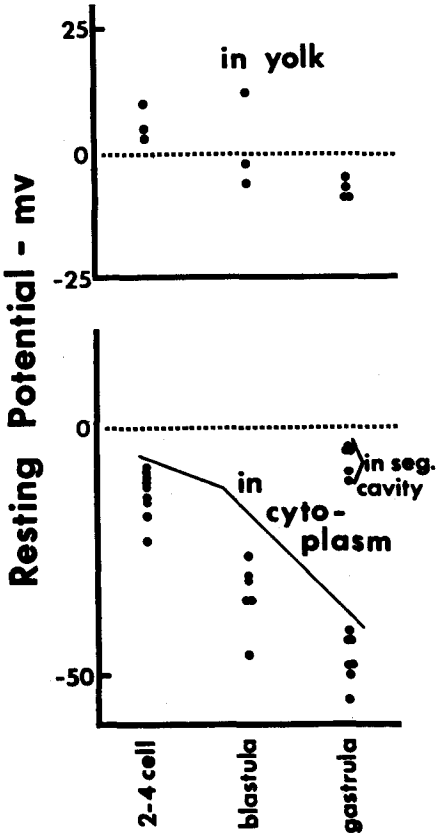

FIGURE 10 Resting potentials at cleavage, blastula, and gastrula stages. The differences between resting potentials in cytoplasm of different stages is significant by the Mann-Whitney U test ($P < 0.01$). In gastrulae the differences between resting potentials in yolk and in cytoplasm and between those in the segmentation cavity and in cytoplasm are similarly significant.

membrane and the external surface of the yolk cell, the yolk membrane must also have come to develop a resting potential such that the yolk was positive to the cytoplasm.

The Leakage Path from Yolk to Exterior

In Fig. 11 *A* and *B* two possible paths are diagrammed whereby current might leave the yolk without passing through the cytoplasm of the embryo proper. The first is leakage around the electrodes penetrating the yolk, and the second is passage across the yolk cytoplasmic layer. A third possibility, that some kind of pore from yolk to exterior exists, can be excluded for lack of a morphological basis.

The first possibility is supported by the following

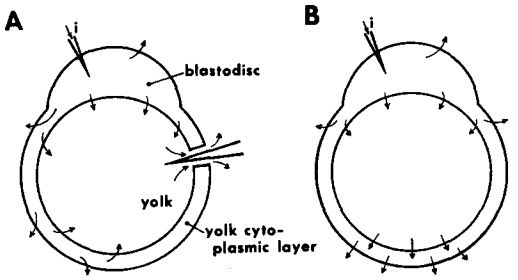

FIGURE 11 Possible current paths from yolk to exterior. *A*. Around electrodes penetrating the yolk. *B*. Across the yolk cytoplasmic layer at the vegetal pole of the cell.

observations. If the yolk was penetrated very carefully with minimal dimpling, the leakage resistance was much greater. In one such experiment, illustrated in Fig. 12 *A'*, the potential in the yolk was only slightly smaller than that in the cytoplasm when current was applied in the cytoplasm. The calculated value of the leakage resistance was 13 MΩ, which is much higher than the usual value of about 0.5 MΩ obtained when electrodes were placed in the yolk rather less carefully (Table I). In several experiments on gastrulae (Fig. 12 *B*, *B'*), electrodes were pushed into the yolk through the enveloping layer and segmentation cavity rather than directly across the yolk cytoplasmic layer. In these cases, when current was applied in the segmentation cavity, the potential in the yolk was identical or nearly so to that in the segmentation cavity. There was little or no leakage path directly from yolk to exterior. Any leak around the electrodes penetrating the yolk must have been into the embryonic compartment rather than directly to the exterior.

In confirmation of the preceding data, the yolk cytoplasmic layer was found to be at very nearly the same potential as the embryonic compartment in the region of the blastoderm. The data in Fig. 13 are from a gastrula in which epiboly was well under way, the enveloping layer having almost reached the equator of the egg. At this stage an electrode was placed in the yolk cytoplasmic layer at the vegetal pole. (In this and similar experiments, the leakage resistance was made fairly low in order that the potentials in yolk and embryonic compartment differed from each other, irrespective of the one in which current was applied.) When current was applied in the segmentation cavity (Fig. 13 *A*, second pulse), the potential recorded by the electrode in the yolk cytoplasmic layer

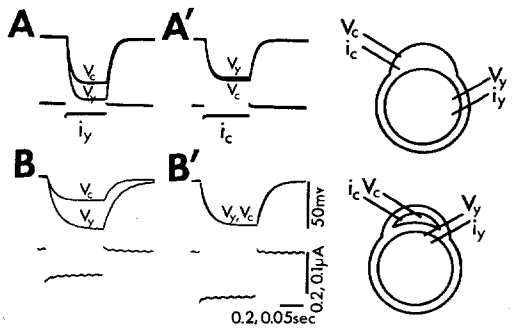

FIGURE 12 Eggs where resistance from yolk to exterior was large. Oscilloscope records on the left, diagrams of electrode placement on the right. Upper two traces, voltages in yolk (V_y) and cytoplasm (V_c) starting from the same base line. Lower trace, current applied in the yolk (i_y in *A*, *B*), in the cytoplasm (i_c in *A'*), or in the segmentation cavity (i_c in *B'*). *A*, *A'*. Data from a two-cell stage where the yolk was penetrated with particular care. R_i was 13 MΩ, much larger than usual. (In Table I, data from this experiment are shown in the first line.) *B*, *B'*. From a gastrula just as epiboly was beginning. Electrodes were pushed into the yolk through the overlying blastoderm. The other two electrodes were in the segmentation cavity. V_y was equal to V_c when current was applied in the segmentation cavity, indicating that the leakage resistance was very high (Table I, the third gastrula).

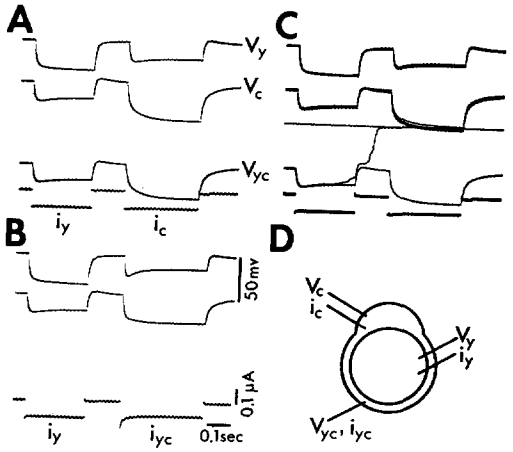

FIGURE 13 Recording from the yolk cytoplasmic layer near the vegetal pole. Data from a gastrula in which the enveloping layer had almost reached the equator of the egg. *A–C*. Oscilloscope records. *D*. Diagram of electrode placement. First trace, voltage in the yolk (V_y). Second trace, voltage in the segmentation cavity (V_c). Third trace (*A* and *C* only), voltage in the yolk cytoplasmic layer and just outside (V_{yc}). Lowest trace, current in the yolk (i_y, the first pulse), in the embryonic compartment (i_c in *A* and *C*) and in the yolk cytoplasmic layer (i_{yc} in *B*). *A*. The potential in the yolk cytoplasmic layer was close to that in the embryonic compartment, and exceeded that in the yolk when current was applied in the embryonic compartment. *B*. When current was applied through the electrode in the yolk cytoplasmic layer, the potential in the embryonic compartment exceeded that in the yolk. *C*. When the electrode was removed from the yolk cytoplasmic layer there was positive shift, indicating that there had been an internally negative resting potential. Note that the applied currents produce no detectable potential outside the egg.

(third trace) was almost equal to that in the segmentation cavity (second trace) and considerably larger than that in the yolk (first trace). The relative sizes of the potentials in yolk and cytoplasm were reversed when current was applied in the yolk (Fig. 12 *A*, first pulse). When current was applied through the electrode in the yolk cytoplasmic layer (Fig. 12 *B*, second pulse), a larger potential was produced in the segmentation cavity than in the yolk. These data prove that current applied in the embryonic compartment could not have been escaping from the yolk to the exterior by way of the cytoplasm in the region of the vegetal pole.

For unknown reasons, the yolk cytoplasmic layer was more difficult to penetrate in the blastula. However, records like those in Fig. 13 could be obtained from this layer several hundred microns from the blastoderm. Although electrotonic spread along the yolk cytoplasmic layer is difficult to calculate exactly, the space constant between flat sheets of the same membrane properties as the yolk cytoplasmic layer would be about 1.3 mm (assuming membrane resistivities of 50 and 100 kΩcm^2 and 5μ thick cytoplasm of 100 Ωcm resistivity). This value is consistent with the observed measurements, considering that the yolk cytoplasmic layer is essentially a closed end conductor, and decrement should be less than in an infinite cable. It is possible, however, that when the leakage resistance is large, as in Fig. 12 *A*, some current applied in the embryonic compartment enters the yolk in the periblast region and then passes out of the yolk at the vegetal pole, as diagrammed in Fig. 11 *B*.

The precise structure of the leak out of the yolk is unknown. The yolk cytoplasmic layer may be greatly compressed at the site of penetration so that there is little electrotonic spread along this

layer. Alternatively, the internal and external membranes may fuse to wall off a channel around the electrode from the yolk to the exterior.

DISCUSSION

Pathways of Communication between Cells

From the electrical measurements it is clear that there are two pathways whereby small ions and other substances can pass between embryonic cells. One pathway is through the extracellular space that comprises the segmentation cavity. The other involves a specialized junctional relation between adjacent cells and is here demonstrated for surface cells and for isolated blastomeres of *Fundulus*. These two modes of communication could each be important in development. Junctional communication provides a "private line" between a cell and its immediate neighbors. For surface cells of *Fundulus* this pathway does not allow communication by means of small ions over distances greater than a few cell diameters because of the low resistance of the cells' inner faces (Figs. 6, 7). In electrical terms, the space constant for electrotonic spread of current is quite short. For larger ions or molecules, communication could be over greater distances, if the junctions were permeable to these substances and the nonjunctional membranes were not. Relatively greater permeability to sucrose (mol wt 342) and Procion Yellow M4RS (mol wt about 500) has been found at gap junctions of the crayfish septate axon (35 and M. V. L. Bennett and P. B. Dunham, Sucrose permeability of junctional membrane at an electrotonic synapse, *Biophys. J.*, in press).

Communication by way of extracellular space could allow signalling over a greater distance than junctional communication. In electrical terms the space constant for current spread in the segmentation cavity is long compared to its diameter. It seems very probable that nutrients from the yolk are released into the segmentation cavity by the periblast rather than transmitted from cell to cell across intercellular junctions. Signalling substances analogous to hormones could also be transmitted by way of the segmentation cavity. Another factor that has been considered important in development is the assessment of position in the embryo (cf. 17). This process might involve the assessment of over-all size through secretion of substances into the confines of the intra-embryonic extracellular space.

From comparison with other tissues, we expect that junctional communication between *Fundulus* cells is mediated by gap junctions, although the apical tight junctions might possibly contribute as well. What are apparently gap junctions have been observed between cells of the enveloping layer in *Fundulus* gastrulae. They have not as yet been seen in blastulae, but this stage has proved more difficult to fix satisfactorily. In principle, coupling could also occur where specialized low resistance membranes were apposed, but remained separated by a "normal" intercellular cleft of 200 A (7). Present electron microscopic techniques do not distinguish between membranes of greatly differing resistivity, for example the outer and inner membranes of cells of the enveloping layer.

For there to be communication by way of the segmentation cavity, there must be little leakage to the exterior. The outer surface membrane meets this requirement, at least for small ions, as is demonstrated by its high resistance. The specific resistance is of the order of 100 $k\Omega cm^2$, a value that is high in comparison to that of most plasma membranes. It is known to be exceeded only in the eggs of several vertebrates developing in fresh water (19), in the wall of the canal of the ampulla of Lorenzini, which is specialized for the spread of electric signals (52), and in the slow muscle fiber of the frog (43).

The inner membranes of the cells of the enveloping layer are of low resistance compared to the outer surface, but they may not be of particularly low resistance compared to membranes in general. The data reported here are consistent with a resistivity as great as 100 to 1000 Ωcm^2.

The high resistance between segmentation cavity and exterior requires also that the intercellular clefts of the enveloping layer be sealed off. The only structures that appear to provide a basis for this barrier are the tight junctions at the apical margins of the superficial cells (51). These junctions are present at all early stages, but in blastulae the extracellular space of the segmentation cavity is so small that electrical recording from it is difficult. Although close appositions completely surround the apical margins of the cells, it has not been shown morphologically that the tight junctions form a continuous band encircling the cells. The electrical measurements allow calculation of an upper limit to the amount of open cleft between cells, if it is assumed that all current leaves the embryo through the clefts, i.e., that the resistance

of the outer plasma membrane of the enveloping layer is infinite. For current flow between segmentation cavity and exterior, the resistance of the cleft, R, would be given by the formula

$$R = \frac{\text{solution resistivity} \times \text{cleft height}}{\text{cleft length} \times \text{cleft width}}$$

If the superficial cells are taken to be hexagonally packed cells, 30 μ in diameter, and the surface area of a gastrula blastoderm is 1 mm^2, there would be a total length of about 10 cm of cleft between cells.[2] If the surface cells are considered to be separated by 100 A over 10 μ of apposition below the apical junctions, and if the solution resistivity is 100 Ωcm, the resistance of this part of the apposition would be about 10 kΩ. This value is small compared to the 0.5 MΩ observed for R_s (Table I), and most of resistance must be ascribed to the apical junctions. If the junctions are 0.5 μ in height and are assumed to be separated by a uniform cleft filled with electrolyte with the same 100 Ωcm resistivity as the bulk solution, the calculated uniform cleft width is 0.1 A. This value is smaller than the crystal radius of the Na^+ ion, and obviously the assumption of bulk solution resistivity does not apply. One can conclude that most or all of the cleft is so narrow that it greatly impedes the flow of the small ions in the bathing solution, and that the width cannot greatly exceed the diameter of these ions over most of the length of the cleft. No more than one-thousandth of the cleft could be occupied by 100 A diameter channels crossing the region of the apical junctions. These electrical measurements thus indicate that the tight junctions in the apical regions are circumferential and seal off most if not all of the extracellular cleft between cells. (A less likely alternative is that the narrowed electron-lucent gap remaining between the apical margins of the cells is filled with insulating material; these regions would in any case have to completely surround the cells.) Similar calculations indicating an occluded gap have been made in respect to the tunicate heart (24) and the canal wall of the ampulla of Lorenzini (52). Thus, electrical measurements provide information like that obtained by use of marker substances in establishing impermeability of circumferential tight junctions (*zonulae occludentes*). Localization to the apical region requires morphological data, but electrical measurements provide information about small ions not yet obtainable by morphological means. Electrical measurements also validate the existence of tight junctions in the living material, a point of considerable value since close appositions resembling tight junctions in some instances appear to be produced as an artifact of fixation (cf. 9, 12).

The demonstration that current can leak around electrodes penetrating the yolk raises the possibility that the actual membrane resistances were much higher than the measured values. Although reduction in resistances was often observed to follow known injury, two lines of evidence suggest that the higher observed values were not greatly in error. First, if an input resistance in yolk or embryonic compartment (or a transfer resistance between them) was measured with a single pair of electrodes, insertion of a second pair of electrodes often did not lead to values different from those measured originally. Second, if current were leaving the egg only at sites of electrode penetration, a detectable voltage drop would be recorded by an external electrode close to these sites. (The potential would be about 2 mv at a distance of 10 μ from a point leak of 10^{-7} A.) In several experiments no such voltage drop was recorded unless a deliberate injury had been made at the penetration site.

The high surface resistance of the *Fundulus* egg contributes to one of its most remarkable features. In spite of the fact that isolated blastomeres and explants of blastoderms deprived of the enveloping layer are highly sensitive to variations in ionic constitution and are rapidly killed by distilled water and sea water, the whole egg undergoes complete development in both these solutions, whether in the chorion (1) or dechorionated (Trinkaus, unpublished data). This wide tolerance of ionic concentrations would require a considerable expenditure of metabolic energy, if it weren't for the low ionic permeability of the surface layer that is evidenced by its high electrical resistance. The electrical results therefore support the concept derived from experimental embryology that the enveloping layer constitutes a protective sac

[2] The number of cells can be taken as the total area, A, divided by the area of a single hexagonal cell, $A/[(3/8)\sqrt{2}\ d^2]$ where d is the major diameter. The circumference of each cell is three times the diameter. Neglecting the edges of the area, the total cleft sength, L, would be half the total circumference, lince the cleft is formed by two apposed surfaces.

Thus: $L = 2\sqrt{2}\ A/d$.

enclosing a stable milieu in which the deep cells develop to form the embryo. The tolerance of different media also indicates a low surface permeability to water as has been shown in the eggs of several teleosts (25, 37, 38) including *Fundulus* (A. Cass, J. P. Trinkaus, and M. V. L. Bennett. Data in preparation).

A relatively impermeable surface barrier may be found in many embryos. In several echinoderms the segmentation cavity is initially open to the exterior, but it becomes sealed off from the exterior at the morula stage (13, 31). The electrical properties of the early newt egg appear to be the same as those of the *Fundulus* egg (20). The resistance between segmentation cavity and exterior has not been measured directly. However, spread between cells was reduced when the surface was opened mechanically, suggesting an extracellular component as well as the remaining component presumably mediated by specialized junctions. A possible criticism of this result is that mechanical injury is known to cause cells to decouple in several different tissues (4, 28, 29, 34). In the absence of morphological data the authors proposed an outer, high resistance membrane external to the plasma membrane of the superficial cells. Development of coupling between isolated blastomeres was observed in the newt, although the degree of coupling was much smaller than usually observed in *Fundulus* and probably too small to account for the coupling *in situ*. The electrical measurements on the egg of the clawed toad do not distinguish between coupling by junctions or coupling by extracellular pathways (42), but presumably these eggs are like those of the newt.

In the chick the resistance of the pathway between extracellular space within the embryo and the albumin has yet to be determined. The measurements showing a low resistance to the exterior were made on isolated embryos with many cut surfaces that opened the intra-embryonic spaces to the bathing medium (41). Since the hen's egg is cleidoic and the embryo develops within a highly controlled environment, the resistance between the exterior and interior spaces could be quite low. In mammals where the embryo develops in the relatively controlled internal milieu of the uterus, there is a "placental barrier" which can prevent passage of vital strains from maternal circulation to embryo (5).

Only in the squid embryo does available evidence suggest that there is little surface barrier, i.e., current passed to the exterior from at least some intra-embryonic extracellular spaces without passing through the superficial cells that were penetrated by microelectrodes. The pathway from interior to exterior is not known. It is unlikely to be between the superficial cells in general, because many of these cells are joined by septate desmosomes (J. M. Arnold, personal communication) that probably seal off intercellular clefts in the same way as do tight junctions. The relatively low resistance to the exterior may in part account for the difficulty of growing the squid embryo outside its chorion (2). Referred to over-all external area, the surface resistivity of the squid embryo is lower than that of the *Fundulus* embryo. Assuming the surface capacitance to be 1 μF/cm, the long input time constant indicates that the actual membrane resistivity is the same as in *Fundulus*, or even somewhat higher.

Properties of the Yolk Membrane

The yolk membrane, like the surface membrane, is of high resistance; and this high resistance may serve a similar barrier function. The low utilization of yolk over several weeks of development, for example, may be possible only because the yolk is confined and separated from cytoplasmic enzymes.

The properties of the yolk membrane have not been evaluated in other embryos whose electrical properties have been studied. Measurements should be made on the chick, which, like the teleost, has a meroblastic egg. In the squid, which also develops meroblastically, a single giant yolk cell contains many small vesicles or yolk platelets that are probably too small to be penetrated by one, let alone two electrodes (3). Electrodes in the single large yolk cell do record almost the same potential throughout (36), but this characterizes the surface and cytoplasmic resistances only and does not disclose the resistance of the yolk platelet membrane. In the newt also, the yolk is contained in vesicles that appear too small for study with microelectrodes (22).

The only other intracellular membrane in animal cells that has had its resistance adequately measured is the nuclear membrane. Its resistivity may be negligible or it may be as great as 1 Ωcm^2, still some five orders of magnitude smaller than that of the yolk membrane of *Fundulus* (27).

In addition to its electrical resistance and capacity, the yolk membrane also generates a

resting potential. This is small at fertilization but increases with time. The gradual increase may be due to a gradual development of semipermeablity. It could also be due to a redistribution of ions. But in the salmon egg the concentration of K^+ in the yolk remains at a high level until long after the stages studied here (18). If the situation is the same in *Fundulus*, the resting potential across the yolk membrane cannot be due to the difference between K^+ concentration in yolk and in cytoplasm. Electron microscopy suggests that yolk is broken down before crossing the yolk membrane, and the yolk membrane probably has transport abilities. Yolk is relatively electron lucent close to the yolk membrane beneath the periblast, and there are no pinocytotic vesicles evident (26). These data lead to the conclusion that intracellular membranes can be similar to extracellular membranes in their passive electrical properties, and perhaps in their transport properties as well.

CONCLUDING REMARKS

The present paper emphasizes two potential functions of junctions between cells. The junctions may allow ions and small molecules to pass between interiors of coupled cells; and when forming a circumferential band between cells of an epithelium (a *zonula occludens*), they may prevent leakage across the epithelium through extracellular space between cells. Both junctional functions may be involved in intercellular communication. The former has been emphasized in recent electrophysiological studies on electrotonic synapses (9), cultured cells (16), and epithelia (30); the latter has been emphasized in morphological work on epithelia (15, 39). As brought out in the introduction there is now reason to believe that these two functions are served by different structures. Indeed, if the junctional area is not very large, significant coupling requires lowered membrane resistivity at the junctions (6), a property not relevant to block of transepithelial movement. It is tempting to suppose that the periodic structure in gap junctions is associated with relatively large channels connecting the cell cytoplasms (35), a prospect made more attractive by the nonspecific permeability of the junctions (8).

The embryological significance of electrical coupling remains to be evaluated. An interesting question is whether the deep cells of *Fundulus* are coupled by junctions and by way of extracellular space as are the superficial cells, or whether they are coupled only by way of extracellular space. The deep cells are, after all, the cells from which the embryo develops.

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