# THE ONSET OF ELECTRICAL COMMUNICATION BETWEEN CELLS IN THE DEVELOPING STARFISH EMBRYO

JOSEPH TUPPER, JOHN W. SAUNDERS, JR., and CHARLES EDWARDS. From the Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203, and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

In many adult tissues electrical measurements have shown the cells to be joined by regions of low electrical resistance, indicating that there is relatively free movement of small ions between them. Similar junctions have been identified electrically in some embryonic tissues (see Ito and Hori, 1966; Potter, Furshpan, and Lennox, 1966; Sheridan, 1968; for reviews, see Loewenstein, 1966; and Furshpan and Potter, 1968). In the newt *Triturus pyrrhogaster* (Ito and Hori, 1966) electrical coupling is found between the two cells resulting from the first cleavage, and thereafter between all blastomeres. It also occurs between all cells in early cleavage stages of the teleost fish *Fundulus* (Bennett and Trinkaus, 1968). However, in the echinoderms *Asterias* (starfish) and *Echinarachnius* (sand dollar) no junctions of low resistance are found between the first two blastomeres (Ashman, Kanno, and Loewenstein, 1964, and Loewenstein, 1966). It is of interest, therefore, to determine if and when electrical coupling does arise between the cells of echinoderm embryos, and whether the appearance of this coupling can be correlated with their developmental properties.

The results of our electrical measurements on eggs of *Asterias forbesi* are the subject of this paper. They show that the daughter cells of the first cleavage are not coupled electrically. Ashman et al. (*op. cit.*) have reported the same result previously, although in their experiments the eggs were treated with urea to soften the fertilization membrane. It has been suggested (Ito and Loewenstein, 1969) that such treatment may have altered the coupling pattern present in the *Asterias* egg. Our studies show this not to be the case. In addition, our studies show that there is no significant electrical communication between blastomeres after the second, third, or fourth cleavages. However, after the fifth cleavage (i.e., at the 32-cell stage), the passage of ion currents between blastomeres occurs, indicating that junctions of low resistance are established by this stage. These findings correlate well with the observations of Wolpert and Mercer (1963) on embryos of *Psammechinus miliaris*, a sea urchin. Their electronmicrographs illustrate the formation of septate desmosomes between cells first occurring at the early blastula stage of this embryo. Septate desmosomes are strongly implicated as the means of electrical communication between cells in other systems (Loewenstein, 1966).

## MATERIALS AND METHODS

Fertilized eggs of *A. forbesi* were obtained according to the procedures described by Costello, Davidson, Eggers, Fox, and Henley (1957), and were held in running sea water until the desired cleavage stages were reached. The eggs were immobilized for recording by a suction micropipette (Fig. 1; cf. Tyler, Monroy, Kao, and Grundfest, 1956) on the stage of a binocular dissecting microscope. All manipulations were carried out at a magnification of 50 ×. Blastomeres were impaled by KCl-filled microelectrodes having resistances of 20–50 Mohms. To achieve impalement, each electrode was advanced against the cell membrane, visibly invaginating it in the case of

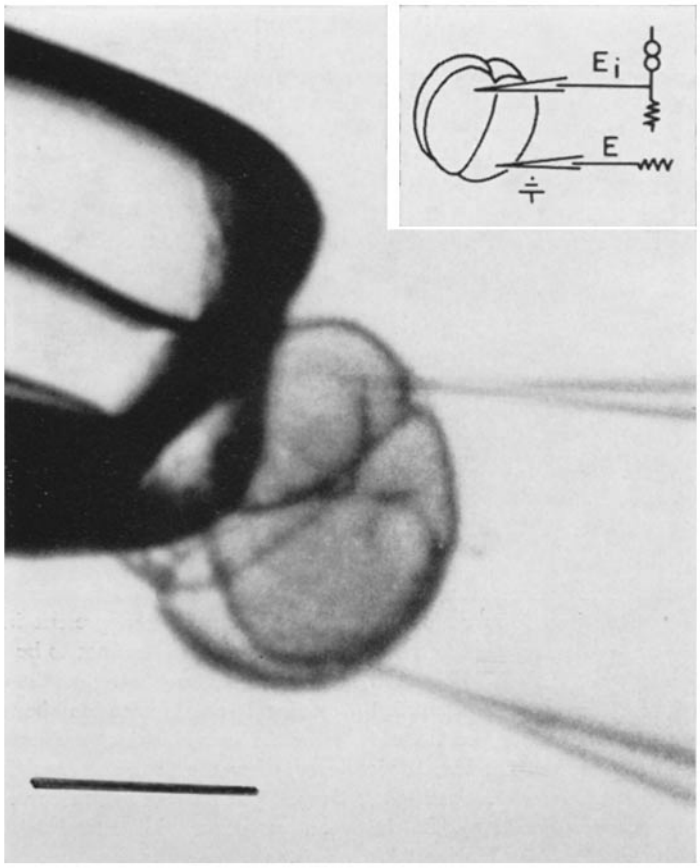


FIGURE 1 An impaled 4-cell stage of the *Asterias* embryo. The cells are held in position by the suction pipet on the left. Note the presence of the cleavage furrows arising for the next division. In several cases records were obtained from cells such as this which underwent normal division during impalement. The recording circuit for this and all other impalements is shown in the *inset*. $E_i$ is the current-passing and recording electrode. The scale is approximately 100 $\mu$.

one-half to one-eighth blastomeres. A slight rap on the table then usually brought about successful penetration, as determined by an increase in negativity of the potential and an increase in input resistance.

To determine electrical coupling, two electrodes, designated as $E_i$ and $E$ (*inset*, Fig. 1), were used to impale separate cells (or the same uncleaved egg). A current was passed through $E_i$ and the membrane voltage drop was monitored by means of two high input impedance negative-capacity amplifiers connected to a cathode ray oscilloscope. Photographic records were taken of the oscilloscope traces for later analysis. The use of electrode $E_i$ for both passing current and recording eliminated the need of a separate electrode for current passage. The system has been previously described (Fein, 1966). It was necessary to use a single electrode since impalements of a single cell with two electrodes in later stages (8- to 32-cell stage), where successful, was followed by a rapid decay of the cell membrane potential and resistance. It was possible, however, to use an independent electrode for passing current with the larger cells of the younger embryos. The voltage drops were 10–30 per cent smaller than those observed where a single electrode was used for passing current and recording. As a consequence, in the single-cell stage there is an apparent lack of isopotentiality observed when using the combination current-passing and recording electrode. This is reflected by the coupling coefficient of 0.7 in Fig. 3. With an independent current-passing electrode the coupling coefficient is approximately 1, as observed by us and others (Ashman et al., 1964). At this stage the membrane resistance is approximately 5 kohm·cm$^2$. If it is assumed that the egg approximates a cylinder of radius 60 $\mu$ with an internal resistivity of 25 ohm·cm, the length constant is

0.7 cm and cannot play a significant role in the voltage difference observed. In experiments with circuits simulating the experimental arrangement, small imbalances of the circuit associated with the current-passing electrode produced alterations only of the voltage drop across electrode $E_i$. The voltage drop across electrode $E$ was constant and dependent only on the magnitude of the resistor and the capacitor (i.e., the membrane) placed in series with both electrodes. This suggests that the increased voltage drops recorded under experimental conditions by $E_i$ may arise due to a consistent imbalance of the system upon impalement of $E_i$. This may be due to some factor, e.g. conductivity change or partial plugging, which is not dependent on the membrane resistance but is additive to the depolarization produced by the membrane resistance.

The combination electrode has been used throughout, and it should be noted that no correction has been applied to either the membrane voltage drops or the coupling coefficients presented here.

## RESULTS AND DISCUSSION

Electrical records were obtained from a total of 42 fertilized and cleaving eggs at stages of 1–32 cells. Typical electrical traces obtained at each stage are shown in Fig. 2. A summary of the electrical measurements is shown in Fig. 3. There is free ion flow between the two electrodes prior to the first cleavage, as shown by the depolarization in membrane voltage recorded by the second electrode when current is passed (Fig. 2 *a*, *b*). The particular egg whose electrical record is shown in Fig. 2 *b* completed its first division while under observation, and the cleavage furrow passed between the indwelling electrodes; the traces reproduced in Fig. 2 *c* were then recorded. The cleavage clearly created an ion barrier between the daughter cells, as shown by the absence of a significant drop in voltage in the record from the second electrode when current was passed by the first. Similar electrical records were obtained in five other cases in which successful impalement of adjacent blastomeres at the 2-cell stage was achieved.

Likewise, there are no indications of ionic communication between blastomeres at the 4-, 8-, and 16-cell stages, as illustrated for typical cases in Figs. 2 *d*, *e*, and *f*. In none of several recordings made at each stage (see legend for Fig. 3) was a significant drop in membrane voltage detected in the recording circuit.

At the 32-cell stage, successful impalement of separate blastomeres was accomplished in four

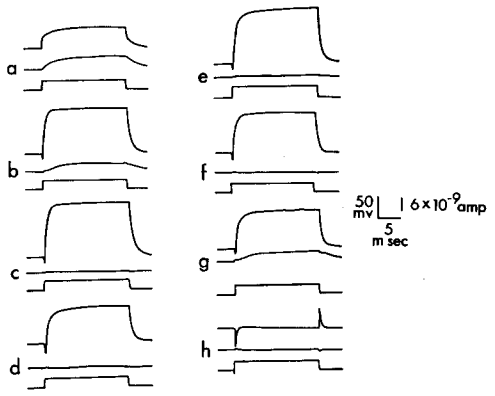


FIGURE 2 Sample records obtained throughout the early cleavage stages in *Asterias*. In all records, the first trace is from the single current-passing and recording electrode. The second trace is from an adjacent or nearly adjacent cell, and the bottom trace is the current pulse. The records are: (*a*) fertile single cell, (*b*) partial first division, (*c*) 2-cell stage, (*b* and *c* are from the same cell), (*d*) 4-cell stage, (*e*) 8-cell stage, (*f*) 16-cell stage, (*g*) 32-cell stage or early morula, and (*h*) a control pulse with the electrodes in the external medium. Note the decrease and subsequent loss of membrane voltage drop in the lower electrode traces of *a* through *c*, illustrating the formation of a resistant membrane between daughter cells. Also note the membrane voltage drop in both electrode records of the early morula (*g*), illustrating the initiation of coupling.

specimens. In each instance the trace from the recording electrode $E$ showed a significant drop in membrane voltage (Fig. 2 *g*) during passage of current.

To assure that the apparent ion flow at this stage was not the result of a flow of current across the surface of the morula, which has been shown to represent a highly resistant barrier in amphibians (Holtfreter, 1943), in all experiments at the 32-cell stage the microelectrodes were used to tease adjacent groups of cells gently apart until a crack extending into the internal cavity was visible between them. The electrodes were then placed in cells which were visibly nonadjacent and as distant as possible from the area of the crack, and records similar to Fig 2 *g* were obtained during successful impalements. In a previous study (cf. Ito and Loewenstein, 1969, Table I) use of a similar technique led to a decrease in the apparent coupling ratio but did not result in the loss of coupling in *Triturus*. It is clear, therefore, that free ionic communication exists at the

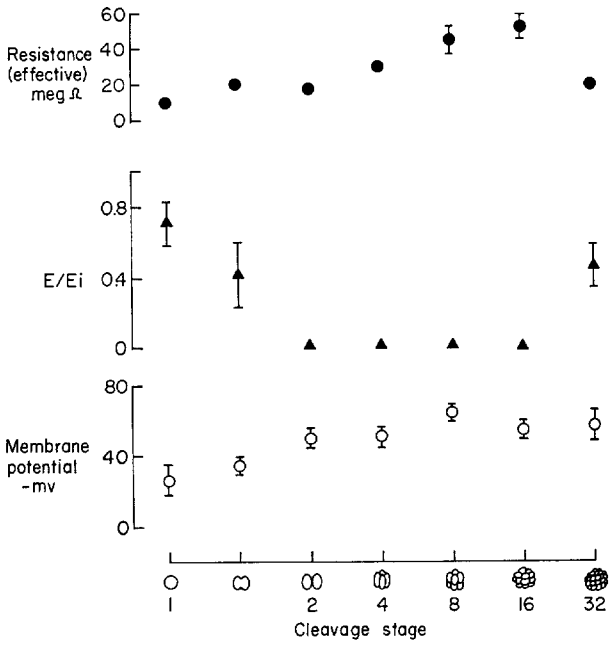


FIGURE 3 The relationship between the coupling coefficient or attenuation factor ($E/E_i$, where $E_i$ is the voltage drop of the cell impaled with the current-passing electrode and $E$ is the voltage drop in the adjacent cell), membrane potential, and effective resistance during the various cleavage stages in *Asterias*. The deviations shown are the standard errors and are less than the size of the symbol where no bar is shown. The number of successful impalements of a pair of cells (each set of impalements being in a different embryo or in an embryo which continued cleavage while impaled) for each stage is as follows: 1 cell, 8; partially cleaved, 9; 2 cell, 7; 4 cell, 6; 8 cell, 6; 16 cell, 5; 32 cell, 4; the cellular division scale is not proportional to time.

32-cell stage between some, it not all, blastomeres.

The 32-cell stage is also characterized by an abrupt drop in effective membrane resistance, $E_i/I$, as illustrated in the upper plot in Fig. 3. The effective resistance shows a progressive increase beginning at the first cleavage (see also Ashman et al., 1964) and extending to the 16-cell stage. The drop at the 32-cell stage probably reflects the appearance of hitherto absent junctions of low resistance, which allow current flow through some or all cells of the blastula and thus reduce the effective resistance. In eggs of *Triturus* (Ito and Hori, 1966) a significant drop in membrane resistance appears upon completion of the first cleavage, and coupling is not lost in that egg when the cleavage furrow passes.

In *Asterias* eggs there also appears to be an increase in membrane potential during the early cleavage stages (Fig. 3, lower plot). A similar increase in membrane potential was observed by Ito and Hori (1966) during cleavage in *Triturus pyrrhogaster*, and by Bentrup (1969) during early development of *Fucus* eggs.

The kinds of electrical measurements reported here for eggs of *A. forbesi*, and by others for different eggs, provide positive evidence that some ionic communication occurs between blastomeres. But the recording methods are not so sensitive as to exclude the possibility that some electrical interaction occurs between blastomeres prior to the stage of observable coupling. It should also be recognized that the pattern of electrical communication revealed by present methods reflects ontogenetic changes in the properties of junctional membranes as well as the degree of intercellular shunting. There also exists a possibility of mechanical interruption of coupling upon electrode insertion due to low cell adhesiveness during the earlier stages. However, no separation of the cells was visible at these stages.

The developmental significance, if any, of the onset of free ionic communication between blastomeres of the echinoderm egg is obscure. Are junctions of low electrical resistance likewise junctions that mediate the passage of morphogenetic information? In starfish and sea urchins normal development requires that there be morphogenetic communication between animal and vegetal halves of the germ. This is shown by the fact that whereas meridional halves isolated during cleavage stages or the blastula form normally proportioned but reduced larvae, isolated animal and vegetal halves do not. The animal half forms only a blastula, with an extended apical tuft, which does not gastrulate; and the vegetal half forms a disproportionate larva, with a large gut and irregular skeletal spicules. Experiments in-

volving the isolation and recombination of blastomeres in sea urchins show that the effective morphogenetic interaction of animal and vegetal halves (Horstadius, 1939) probably occurs much later than the 32-cell stage, when we find the onset of electrical communication in blastomeres of the starfish. Nevertheless, it will be of interest to determine whether in the starfish and other echinoderm embryos there is an ontogenetic pattern in the origin of junctions of low electrical resistance that can be related to the axial organization of the embryo. This will require further experiments in which measurements are made with electrodes that have impaled blastomeres in known position with respect to the planes of successive cleavage furrows.

This research was supported by grant No. NB07681 and HDO 3734-02 from the National Institututes of Health, and grant No. GB6638X from the National Science Foundation.

Dr. Tupper thanks the Grass Foundation for a summer fellowship at The Marine Biological Laboratory.

*Received for publication 5 December 1969, and in revised form 24 January 1970.*

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