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7. Identical cardboard disks from which appropriate portions had been cut and removed were placed above and below two petri dishes. The disks were attached to a rotatory motor producing one revolution every five minutes. Unfiltered light from two American Optical illuminators (both housing a General Electric bulb model 1594 operated at 7.5 v) provided the illumination for each dish of animals during the 75-second light period. The light sources were placed 20 cm above and below each of the dishes. The dishes were placed so that the cut-out disks exposed one dish to the light leaving the other in relative darkness (approx. 55 lumen/m^2).
8. The homogenate of *A. salina* was prepared from a dense 1-ml suspension of *A. salina* in 5 ml of distilled water. The solution was treated for 1 minute with sonic oscillations and centrifuged at 3000 rev/min for 5 minutes. Samples of 1 ml of the supernatants having a total protein content of 5.8 mg/ml were diluted tenfold and samples of 1 ml of this solution were administered to the hydra.
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10. N. B. Rushforth, unpublished observations.
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13. The animals were shaken at 105 rotations per minute on a Clay Adams Model A 2273 Yankee Variable Speed Rotator.
14. Inhibition of contractions to mechanical agitation also occurs if the stimulus consists of poking the hydra with a dissecting needle. Inhibition of contractions is thus not specific to the mechanical stimulation used, although that applied by means of a rotator provides a more uniformly reproducible stimulus.
15. N. B. Rushforth, in preparation.

16. This research was supported in part by grants from the National Science Foundation and National Institutes of Health.

26 May 1964

Intercellular Electrical Coupling at a Forming Membrane Junction in a Dividing Cell

Abstract. *Ion communication between the halves (blastomere) of a dividing cell* (Asterias forbesi *egg), as determined electrically, diminishes progressively during division as a cell membrane junction forms at the plane of cleavage. Virtually all communication is lost towards the end of division by the time the first intercellular space becomes continuous with the exterior. Resting membrane potentials in both cell halves are essentially equal and constant throughout division.*

During division of egg cells, a cell junction forms at the plane of cleavage. The present study concerns the electrical intercellular coupling as a membrane barrier to ions develops at the junction.

We selected for this study the dividing egg cell of the starfish (*Asterias forbesi*) which offers several advantages. Its plane of cleavage can be predicted at an early stage of cell division; its plasma membrane can be impaled without apparent injury with microelectrodes; and it is large and transparent, so that the position of a microelectrode inside the cell can be clearly viewed and controlled under a microscope (Fig. 1).

The cell was fertilized in vitro, bathed for about 5 minutes in an isotonic urea solution (1M in urea, 0.1M in phosphate buffer, pH 8) to soften and to induce swelling of its fertilization membrane, and impaled with three microelectrodes. Square pulses of current could thus be passed through the cell membrane, and the resulting changes in membrane voltage (time $\rightarrow \infty$) could be measured across the cell membrane on either side of the plane of cleavage (Fig. 2). This arrangement gives continuous information on the intercellular electrical coupling, that is, on the fraction of the current that passes from cell to cell across the plane of cleavage. Moreover, it gives the electrical resistance of the mother cell and, after completion of cell division, the resistance of the daughter cells. The resistance of the urea-treated fertilization membrane is negligible. This resistance, measured directly with microelectrodes placed in the space between fertilization membrane and cell membrane, was in all cases three orders of magnitude lower than the resistance of the cell membrane. Both membranes presented no appreciable rectification nor signs of excitation over the entire range of current used. Microelectrodes of very small tip diameters were employed (*1*). With these electrodes, there were no signs of current leakage, and some of the cells continued division well into the blastula stage. The results reported here are from such cells.

Figure 2 illustrates the results of a typical experiment in which measurements were made throughout the process of cell division, from the earliest stages of cleavage to its completion. At the onset of cleavage, the changes in membrane voltage are about equal in the two cell halves. As cleavage proceeds, the voltage decreases progressively in the half opposite to the current electrode, falling rather abruptly

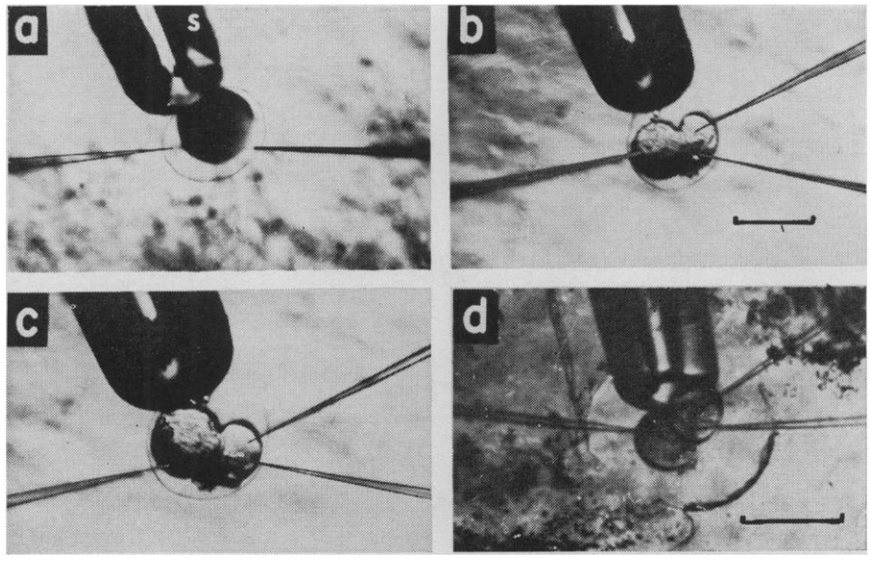

Fig. 1. A dividing egg cell. (*a*) The cell about 95 minutes after fertilization (16°C) in the process of being impaled by two microelectrodes. Its swollen fertilization membrane is sucked onto a glass capillary (*S*) (*5*) to hold the preparation. (*b, c, d*) The preparation at various stages of cleavage with three microelectrodes in intracellular position. Calibration lines for upper and lower rows are 100 μ.

below detectable values as cleavage is completed in this urea-treated preparation. This happens at a time when the two daughter cells become separable by micromanipulation, probably at the time of the appearance of the first continuous intercellular space. The resting potential across the cell membrane is equal on either side of the plane of cleavage and is rather constant throughout all phases of cell division.

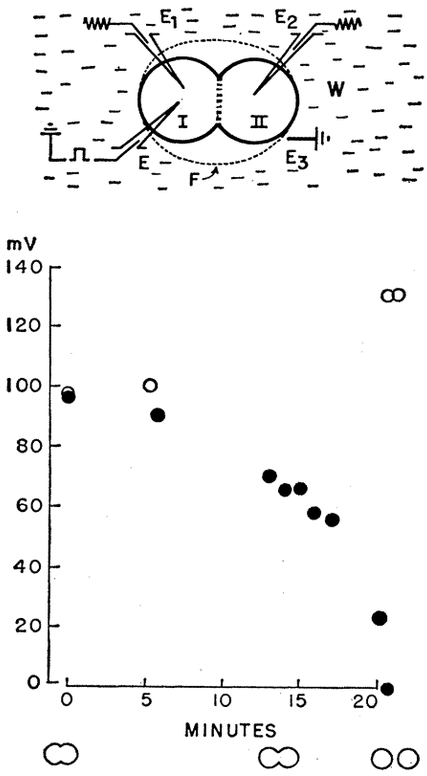

Fig. 2. (Top). Electrode arrangement. Three microelectrodes are inserted into a dividing cell, one (E) to pass square pulses of current across the cell membrane, and the others (E_1 and E_2) to record the resulting changes in potential across the cell membrane of cell halves I and II on either side of the plane of cleavage. E_3 is a low-resistance electrode in common with the current-passing and potential-recording circuits placed in the sea water (W) bathing the preparation. Potentials of E_1 and E_2 are fed into separate beams of a cathode-ray oscilloscope through d-c amplifiers with impedance-matching electrometer tubes at the input stages. The fertilization membrane (F) lies in series with the cell membrane, but its resistance is negligible. In the case illustrated on top, it was 3.6 kohm as against 2190 kohm, the resistance of the cell membrane. (Bottom) Electrical coupling during cleavage. Abscissa: time after onset of cleavage; time zero, at earliest sign of a cleavage furrow. Ordinate: changes in membrane potential in cell halves I, (open circles) and II (closed circles) due to inward and outward currents of 4.5×10^{-8} amp. The rise in potential recorded in I at the end of cleavage (20 min) is less than the rise in potential expected from the smaller surface area of the daughter cell. The resting potential across the membrane of this cell is 43 mv (cell interior negative) in both halves and remains constant within 2 mv throughout cleavage. Temperature 16°C.

A simple explanation of the result is that the decrease in voltage is determined chiefly by (i) the resistance across the cleavage plane, which is eventually to become the cell-to-cell resistance, and (ii) the resistance along the region which is eventually to become the intercellular space. This is perhaps best made clear with the aid of an equivalent circuit of the kind given in Fig. 3, in which these factors are represented by r_c and r_s, respectively. During the first phase, we consider r_c as the primary variable. At first, there is full protoplasmic continuity across the cleavage plane (r_c is at a minimum, and the currents in either cell half are about the same). As cleavage proceeds, r_c rises progressively, and a progressively decreasing fraction of current reaches the half opposite to the current-passing electrode. This phase corresponds with a progressive diminution in the effective cross-sectional area of the protoplasmic bridge at the level of the forming membrane junction.

A simple possibility is that the effective protoplasmic bridge diameter decreases linearly with time, leading to a nonlinear decrease in current and attenuation of voltage. During this first phase, which lasted about 15 to 20 minutes in most cells (15° to 17°C), we consider r_s to be relatively constant and of a similar high order of magnitude to r_o, the resistance of the cell membrane facing the outside. At the end of this phase, although the cell-to-cell resistance is high, there is still some electrical coupling between cells. In the second and final phase, r_s enters into play as a shunt as it falls steeply with the completion of the intercellular space, and becomes the determining factor of voltage attenuation. At this time, virtually all coupling is lost.

This interpretation of results follows a model of intercellular coupling developed recently from measurements in certain nondividing cells in which r_c and r_s are constants and independently determinable (*2*). Indeed, the electrical coupling during the first phase of cell division here resembles closely that existing between certain epithelial cells in which there is free cell-to-cell diffusion of ions (*2*) and a strong barrier to diffusion along the intercellular space (*2, 3*), while that of the second phase is similar to that existing in general between large nerve and skeletal muscle fibers with wide intercellular spaces.

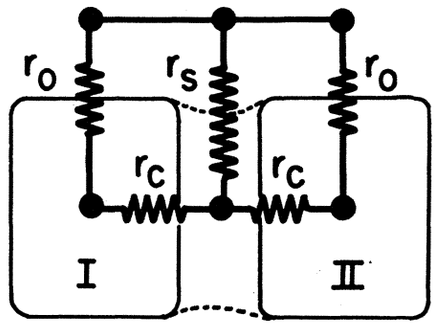

Fig. 3. Equivalent circuit diagram of coupling in a dividing cell.

Another finding of interest concerns the membrane resistance during cell division. Direct resistance measurements were made in the cell at the onset of cleavage, and in the daughter cells, fully separated, at the end of cleavage; the area of the surface membrane was determined from light-microscope measurements of the major and minor cell diameters. The resistance of unit membrane area in the daughter cell immediately after cleavage was invariably smaller than that in the mother cell. For example, in the case illustrated in Fig. 2, the resistances were 544 and 762 ohm cm^2, respectively. Whether this higher permeability resides in the newly formed membrane alone, or reflects differentiations of the old membrane portions as well (*4*) is not resolvable with the present method.

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