Electrical Characteristics of *Triturus* Egg Cells during Cleavage

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ABSTRACT The membrane potential in the blastomeres of dividing *Triturus* egg cells increases progressively from the first cleavage to the late morula stages. Both the animal and vegetal poles show the same increasing trend in potential; there is no significant potential difference between them. Upon first cell cleavage, the total resistance of the egg cell surface in contact with the exterior decreases to about one-tenth of its value before cleavage, and then remains rather constant up to the late morula stage. The specific resistance of this membrane surface drops rather abruptly upon first cleavage, and rises progressively during the morula stage. The resistance of the junctional membrane surface of the blastomeres, that is, the membrane formed at the former planes of cleavage, is small in relation to that of the cell surface in contact with the exterior. As a result, the blastomeres are electrically coupled throughout all stages of embryonic development examined.

Since Backman and Runnström (1912) showed that the osmotic pressure of *Rana* eggs diminishes at the time of fertilization, the permeabilities to water and water-soluble substances of amphibian eggs have been studied under various conditions (Krogh, Schmidt-Nielsen, and Zeuthen, 1938; Picken and Rothschild, 1948; Holtfreter, 1943; Kusa, 1951; de Luque and Hunter, 1959; de Luque, Hunter, and Hunter, 1961); and the permeabilities have been correlated with activation processes (Maéno, 1959), and with oxygen consumption (Løvtrup, 1962 *a*, 1962 *b*, and 1963).

Renewed interest in developmental aspects of cell permeability was stimulated by the work of Kanno and Loewenstein (1963) who showed that the membrane potential and the specific resistance of amphibian egg cells increase progressively during development in the oocyte stages. The present study concerns changes of this kind at later stages of development. It deals with changes after fertilization, including those following egg cell cleavage. It concerns in particular the membrane resistance at the plane of cleavage, which in preliminary experiments appeared to be smaller than that of the

rest of the cell surface (Ito, 1962 *b*). A further objective of the present study was to examine possible potential gradients along the egg polar axis during development, such as reported by Dorfman (1934) and Flickinger and Blount (1957).

MATERIALS AND METHODS

Fertilized eggs of the Japanese common newt (*Triturus pyrrhogaster*) from the following developmental stages were used: eggs before cleavage; 2-cell, 4-cell, 8-cell, 16-cell stages; early morula, middle morula, and late morula. The jelly layer surrounding the egg was removed with De Wecker scissors in Holtfreter's solution (1943); care was taken to avoid injury to the eggs. The chorion was left in place.

The general arrangement employed for electrical recording is illustrated in Fig. 1*A*. Two microelectrodes filled with 3 M KCl were inserted into the egg. Electrode tip

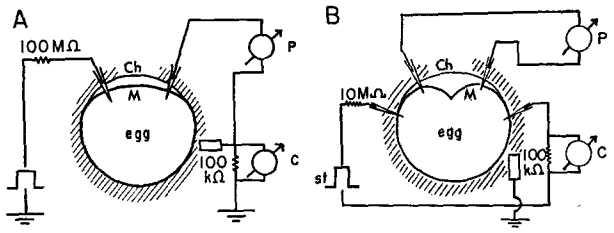

FIGURE 1. Diagrams of electrical setup. *A*, arrangement for measurements of membrane resistance and voltage attenuation. For attenuation measurements the microelectrode is moved to different positions within the egg or to different blastomeres. *B*, arrangement for resistance measurements across the cleavage plane. *Ch*, chorion; *M*, egg cell membrane; *P*, potential recording; *C*, current recording. Preparation is in a physiological salt solution.

resistances ranged from 20 to 50 Mohm. One of the electrodes was used for passing rectangular pulses of current variable in magnitude and duration; and the other electrode, for recording resting membrane potentials, and potential changes produced by the current. The latter electrode was connected to a cathode ray tube through a high impedance negative-capacity amplifier. The current was measured across a 100 kohm resistor in series with the current-passing circuit. The currents used were on the order of 10^{-8} A; their duration was 3 sec for the eggs before cleavage, and 0.5 to 1 sec for the eggs after start of cleavage. One of the following two methods of recording was used in most cases. In one, both the recording and the current-passing electrodes were in the same blastomere (arrangement 1). In the other, the electrodes were in different blastomeres (arrangement 2). In a few experiments, methods 1 and 2 were combined by using three microelectrodes for simultaneous comparison of electrotonic potentials in different blastomeres (see top inset of Fig. 5).

For determinations of the resistance across the cleavage plane, an arrangement of four intracellular microelectrodes was used, as shown in Fig. 1*B*. Current pulses of 100 msec duration and up to 10^{-6} A intensity were passed between two microelectrodes

of 10 to 20 Mohm resistance, isolated from ground; and the resulting changes in membrane voltage recorded across the forming or already formed junctions of different blastomeres. Current direction was alternated to minimize effects of polarization.

The present egg material was easily penetrated by the microelectrodes and offered in this respect a more favorable material than echinoderm (Tyler, Monroy, Kao, and Grundfest, 1956; Hiramoto, 1959) or *Oryzias* eggs (Ito and Maéno, 1960).

RESULTS

Membrane Potentials When the recording electrode established contact with the chorion, a potential of 10 to 20 mv, negative with respect to the external solution, was observed (Fig. 2 *a*). (Polarity of all potentials is expressed hereafter with respect to potential of external solution.) The potential attained its maximum before passing through the chorion. After penetration of the chorion, the potential reverted to zero. The magnitude of the potential

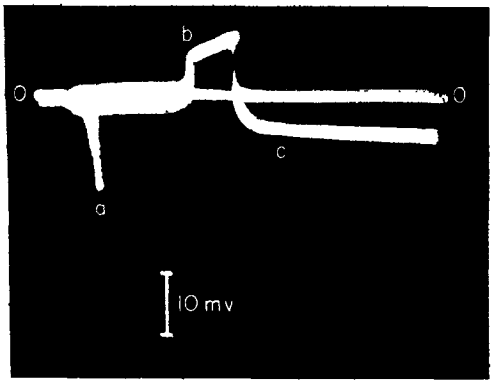

FIGURE 2. Potential changes (downward, negative with respect to outside) upon progressive electrode advancement through the surface of an egg before cleavage. See text.

across the egg cell membrane proper was, therefore, not affected by the chorion contact potential. As the electrode advanced towards the egg cell membrane, a progressively increasing positive potential developed (Fig. 2 *b*). This potential was probably the result of mechanoelectric effects within the electrode tip, due to mechanical strain.

Upon penetration through the ooplasmic membrane, the potential suddenly changed polarity (Fig. 2 *c*). This coincided with the appearance of electrotonic potentials, when arrangements 1 or 2 (see Methods) were used, and gave assurance that the cell membrane had been penetrated.

Table I summarizes the membrane potentials so obtained with electrodes located in the animal area and, from the 8-cell stage on, in both the animal and vegetal areas. Membrane potentials increase progressively from 5 to 11 mv in the stages before cleavage, to values as high as 65 mv in the late morula stage. Both the animal and the vegetal area show the same increasing trend

in potential. The average potential was somewhat lower at the vegetal area than at the animal area; but the differences are not statistically significant.

Egg Surface Resistance and Capacity Fig. 3 illustrates a typical current-voltage relation in the fertilized egg before cleavage. At no instance, was a

TABLE I

MEMBRANE POTENTIALS (MILLIVOLTS)* AT ANIMAL AND VEGETAL POLES DURING DEVELOPMENT

Stages	Animal area	No. of eggs examined	Vegetal area	No. of eggs examined
1-cell	5.6±2.8	24		
2-cell	15.7±6.5	15		
4-cell	20.3±7.1	21		
8-cell	22.6±4.5	10	21.3±3.3	12
16-cell	31.1±9.2	21	28.3±9.6	19
early	35.3±9.5	28	33.4±9.3	27
Morula middle	41.1±9.1	13	37.8±11.1	13
late	53.8±10.1	15	48.6±13.5	14

* Mean values with standard deviation.

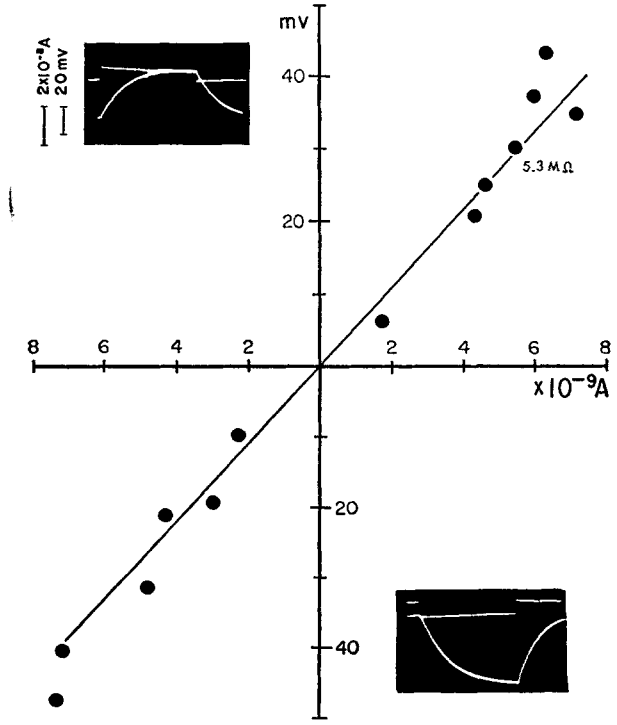

FIGURE 3. Current-voltage relation of a fertilized egg before cleavage. Inward current, downward; outward current, upward. Insets give examples of oscillograph recordings of membrane current and voltage. Current pulse duration, 3 sec.

resistive voltage step found at the level of the chorion. The resistance of the chorion seems to be negligible, and the current-voltage relations to reflect entirely properties of the egg cell membrane. The slope gives the effective surface resistances which ranged from 1.8 to 10 Mohm for outward, and 1.8 to 13 Mohm for inward currents.

Right after spawning, the effective surface resistance of the fertilized egg averages 5 Mohm, which corresponds to a specific membrane resistance of 630 kohm cm^2. These resistances are in the same range as those of *Bufo* eggs (Maéno, 1959) and *Oryzias* eggs (Ito and Maéno, 1960; Ito, 1962 *a*). With further development, the total effective surface resistance undergoes changes which are summarized in Fig. 4. Upon first cell cleavage, the effective re-

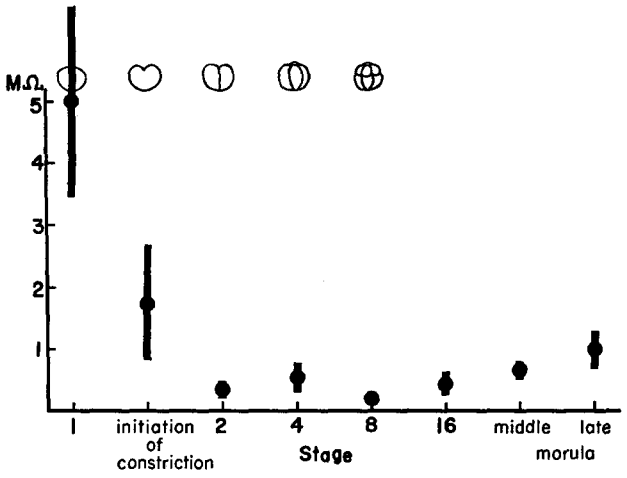

FIGURE 4. Total (effective) resistance during development. Mean values with standard deviations. Distances on abscissa are not proportional to time.

sistance drops markedly and out of proportion with the increased surface area. From the 2-cell stage on, the effective resistance remains rather constant up to the morula stage, and rises then during this stage. During all this time, the cell surface area increases continuously. The exact surface area during the later morula stage could not be determined. However, the trend of the change in specific surface resistance is clear. This drops rather abruptly upon first cleavage, and then increases progressively during the later morula stage.

The membrane capacitance ranges from 0.7 to 1.0 μf/cm^2 and is rather constant throughout development.

Resistance across the Cleavage Plane Fig. 5 illustrates the results of an experiment in which current is passed through one cell in the morula stage and the resulting electrotonic voltages are recorded in two blastomeres 1 mm apart. (See Fig. 5, top.) The most striking result is that the voltage attenu-

ates so little from one blastomere to another. In the example of Fig. 5 the difference in voltage amounts to only 7% for outward current and 18% for inward current. Thus, the resistance must here reside largely in the cell surface in contact with the exterior; the resistances of the surfaces at the level of the junction between blastomeres, i.e. the resistance across the former cleav-

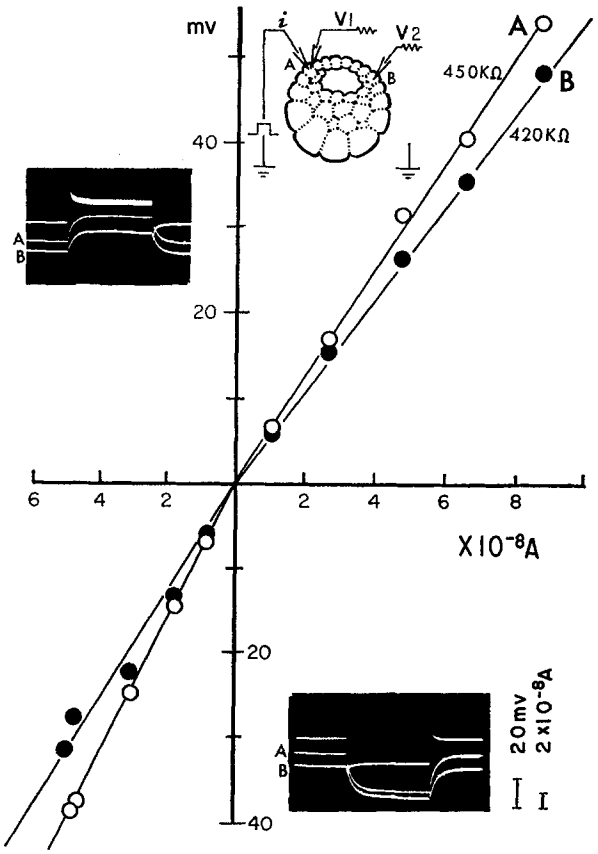

FIGURE 5. Simultaneous current-voltage relations in two blastomeres of the morula stage. Insets give examples of membrane current and voltages. Current pulse duration, 1 sec.

age planes referred to as *junctional membranes*, are small by comparison. (For a full experimental and theoretical analysis of a similar situation in a somatic cell system, see Loewenstein and Kanno, 1964; and Loewenstein et al., 1965.)

The relatively low resistance across the junctional membranes is shown particularly clearly by direct resistance measurements of the arrangement shown diagrammatically in Fig. 1*B*. A result is shown in Fig. 6. After completion of the first cell division, at a time when the junctional membranes are fully formed, the resistance across the cleavage plane is on the average

23×10^3 ohm as against 300×10^3 ohm at the nonjunctional surface membrane. An estimate of the specific resistance of the junctional membrane, obtained by treating the situation of current flow between blastomeres as in a conducting cylinder, gives values of 550 ohm cm^2.

The above kinds of measurements were done only in the outermost layer of blastomeres. No attempt was made to record from more deeply located blastomeres of the morula, since we feared that this might injure the membranes of the cells in the outer layer.

DISCUSSION

Values of membrane potential are now available for several stages of egg cell development. The measurements of Kanno and Loewenstein (1963) cover a

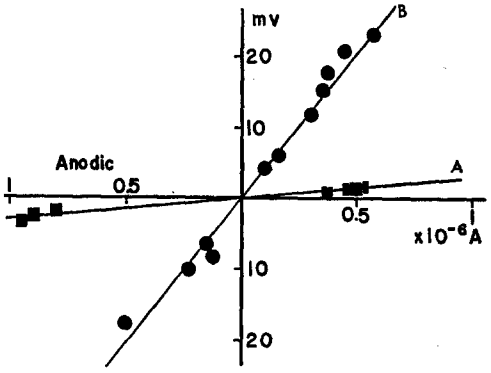

FIGURE 6. Membrane resistance before (A) and after (B) first cleavage.

wide growth span in oocytes; Maéno's (1959) measurements encompass immature, mature, and activated eggs; and the present ones cover the span from the end of fertilization to the late morula. Thus an attempt may now be made at piecing the various data together and giving a rough idea of the time course of membrane potential over a wide span of development. The data come from different species; but these are phylogenetically close enough, and the values overlap sufficiently to make such an attempt worthwhile, at least, in providing an idea of the major trends. The attempt is made in Fig. 7. The resting potential increases progressively with growth in the oocytes (Kanno and Loewenstein, 1963), reaches a peak in the immature egg, falls rapidly to a low value in the mature egg (Maéno, 1959), to rise again progressively after the first cell division.

Dorfman (1934) has reported that a potential difference exists between vegetal and animal poles in eggs of *Rana temporaria* and *Rana arvalis*. Dorfman's observations, made at a time when microelectrode techniques were not yet developed, rely on measurements with electrodes of 20 to 30 μ tip diameter.

These are too large to prevent injury and leakage artifacts. Our measurements show no significant differences in potential between the poles.

Ashman, Kanno, and Loewenstein (1963) made the first studies of electrical resistance across the cleavage plane of a dividing cell. They observed a progressive increase in resistance across this plane during the first cell division in *Asterias* egg cells. In the early stages of cleavage, these authors found good electrotonic coupling between the blastomeres; but towards the end of cleavage, when the junctional membranes are fully formed, the electrical coupling diminished below detectable levels. Recent work by one of us on dividing *Echinarachnius* egg cells confirms in all details this pattern of electrical coupling (Ito and Loewenstein, 1965). The pattern in the present results on the cleav-

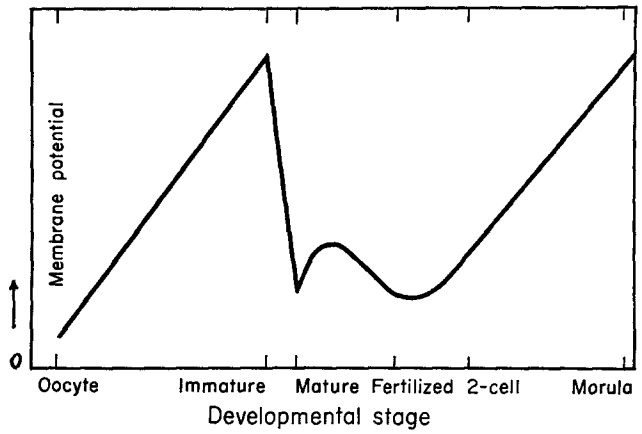

FIGURE 7. Diagram of changes in membrane potential during development of amphibian eggs. Curve drawn on the basis of data from oocyte stages of *Xenopus* (Kanno and Loewenstein, 1963); from egg stages of *Bufo* (Maéno, 1959) and *Triturus* (present results).

ing *Triturus* egg is different. Here electrical coupling is detectable at all stages. However, this does not necessarily mean that the permeability properties of the junctional membranes are basically different from those in *Asterias* or *Echinarachnius*. The difference in the pattern of electrical coupling, as revealed by the method of measuring voltage attenuation, can reflect differences (*a*) in resistivity of the junctional membranes; (*b*) in resistance of the nonjunctional membrane surfaces; and (*c*) differences in shunting by the intercellular medium. It is not possible, at present, to distinguish among the alternatives.

The close electrical coupling between cells at all embryonic stages examined is, however, of interest in itself. This opens the possibility that embryonic cells communicate through low junctional resistances. Communication of this sort has been shown to exist in a wide variety of adult epithelial cells where molecules large enough to carry genetic information flow from cell to cell (Loewenstein, 1966).

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