Electrical Signs of New Membrane Production
during Cleavage of *Rana pipiens* Eggs

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**Abstract** *Rana pipiens* eggs dividing normally in diluted Ringer's solution show an increase in transmembrane potential inside negative, a decrease in resistance, and no change in total surface membrane capacitance at the appearance of a division furrow. Furrows of eggs in solutions with the tonicity of full Ringer develop partially, then regress so that the surface is again spherical. The potential and resistance changes are greater and substantial increases in capacitance occur when furrowing is so inhibited. It is proposed that the electrical changes at division are due to the introduction of new plasma membrane, between the blastomeres, having selective permeability to K and a low resistance compared to the outer spherical membrane. A narrow gap between blastomeres limits current flow through new membrane during normal division. A direct exposure of new membrane to the bathing medium when furrowing is disrupted results in larger changes in potential and resistance and permits the capacitance of new membrane to be detected.

**Introduction**

This study deals with the electrical correlates of cell division in the egg of the frog, *Rana pipiens*. Electrical changes can be expected to occur since the area of the plasma membrane must increase at some time during cytokinesis. An increase in area would be expected to increase the capacitance and decrease the resistance of the plasma membrane. In addition, a change in the transmembrane potential might be expected if the selective permeability characteristics of the total membrane for the various ion species present were altered.

Previous work (Schectman, 1937; Waddington, 1952; Selman and Waddington, 1955; Dan and Kuno-Kojima, 1963; and Zotin, 1964) suggests that cleavage in the amphibian egg takes place by an active growth of membrane within the division furrow. The furrow, once initiated, is self-propagating and eventually divides the egg by means of a narrow slit bordered by the two apposing plasma membranes of the blastomeres.

A question to be asked of an electrical analysis is: where on the surface of the egg and at what stage in the development of the furrow does new mem-
brane appear? Also, what are the ionic permeability characteristics of the new membrane, and when does membrane selectively permeable to potassium or other ions appear?

The evidence presented here indicates that new membrane is located specifically within the division furrow. Further, it is suggested that the new membrane is selectively permeable to potassium the moment it appears on the surface and that it has a low specific resistance compared to that of the spherical preexisting membrane. The results also suggest that the permeability characteristics are unstable and that a spontaneous loss of potassium selectivity and increase in resistance can occur.

A preliminary report of this work has appeared (Woodward, 1965).

METHODS

Electrical Measurements

Microelectrodes with resistances of about 5 megohms and tip potentials of less than 3 mv were used, with calomel electrodes at the liquid-metal junctions. The bath was connected to ground via a calomel electrode with a saturated KCl agar bridge. Cathode followers with grid currents less than $10^{-10}$ amp were used to detect voltages, which in turn were monitored by a paper recorder and an oscilloscope. Drift in the system was less than 1 mv per hr and the rise time was less than 100 μsec.

Two microelectrodes were inserted simultaneously into an egg to pass currents and measure voltages across the plasma membrane. A system was arranged to pass a 2 sec pulse of inward membrane current, usually $-5 \times 10^{-8}$ amp, through one of the electrodes at 1 min intervals. Resistance was calculated from the IR drop at the end of a pulse. (Resting potentials were recorded with both electrodes between the current pulses.) Plotted values of resistance invariably fell along smooth lines, which are employed without loss of information in the figures. Some rectification was evident, since equal and opposite currents might typically hyperpolarize 20 mv or depolarize 16 mv. The changes in resistance of interest are large so that the single measurement of a chord resistance is adequate.

The capacitance of the plasma membrane was determined from photographs of the oscilloscope records by dividing the current injected by the initial rate of voltage change measured during the first 2 msec of the pulse. This simple method of measuring capacity was aided by the long time constants involved (typically 10-200 msec). The accuracy of a single capacity measurement was 5% in the best and 20% in the worst cases reported.

The blastomeres of the eggs during all the stages studied here were connected electrotonically; voltage changes due to a current pulse were equal in all cells (Ito and Hori, 1966). The values for resistance and capacitance therefore correspond to lumped properties of all the plasma membrane of the embryo that admits current flow from the cytoplasm to the bathing medium. The results presented here are confined mainly to the events occurring during the first three division cycles when synchrony of division occurs.
Bathing Media and Their Effects

The saline solutions denoted "full Ringer" contained 120 mM Na, 121 mM Cl, 2.5 mM K, 1.8 mM Ca, 2.15 mM HPO$_4^{2-}$, and 0.85 mM H$_2$PO$_4^-$. Solutions designated as fractions of full Ringer were made by mixing appropriate portions of full Ringer with distilled water, or with distilled water with sucrose added. Variations of Na, K, Cl, and SO$_4^{2-}$ are described in the legends to the figures. Experiments were performed at room temperature (20–25°C).

Frog eggs do not divide successfully when placed in full Ringer (Morgan, 1906; Needham, 1963; Morrill and Watson, 1966). The surface constrictions of the first furrow begin and the egg looks normal for a few minutes. Soon, furrowing ceases, the constrictions relax, and the surface again becomes flat. Similar attempts at division may continue for three to five cycles. A similar inhibition of cleavage is observed in 300 and 400 mM sucrose or in $\frac{1}{2}$ Ringer with 150 mM sucrose added. Eggs after 24 hr in $\frac{1}{2}$ or $\frac{1}{10}$ Ringer develop a black cap of cells on top of an uncleaved vegetal hemisphere. $\frac{1}{10}$ Ringer is the upper limit for normal development. While the mechanism of the inhibition is unknown, the phenomenon has led here to a useful comparison of electrical properties of eggs dividing normally in dilute solutions with properties of eggs with failure of division.

Handling of Eggs

Female frogs (The Lemberger Co., Oshkosh, Wis.) were made to ovulate by injecting one female frog pituitary into the abdomen and 5 mg progesterone in 0.2 ml corn oil under the skin of the back. Eggs obtained 1 or 2 days after the injections were fertilized by exposing them to a sperm suspension (the testes from one frog broken up in 15 ml of $\frac{1}{10}$ Ringer) which was prepared at least one-half hour in advance. The eggs were kept in $\frac{1}{10}$ Ringer until about 5 min before the microelectrode impalement. In most experiments the outer jelly layers were then removed using fine forceps to permit easier penetration by the electrodes. When comparable experiments were done, eggs with and without jelly gave similar results.

The eggs were transferred from $\frac{1}{10}$ Ringer into glass or plastic Petri dishes containing the solutions to be tested and viewed through a dissecting microscope. The tips of the microelectrodes were aimed perpendicular to the surface, thrust simultaneously into the egg, and adjusted at about 100 µ beneath the surface of the plasma membrane of the animal hemisphere. The electrodes were sometimes spontaneously ejected out of the egg if the tips were placed too close to the surface.

Irregular potentials associated with changes in electrode resistance were encountered at the jelly layers and vitelline membrane. An abrupt shift in potential, a disappearance of dimpling, and electrotonic potentials appeared as the tips of both electrodes penetrated the pigmented surface of the egg. No significant potential difference was detected between the external medium and the perivitelline space. The resistance and potential measured are across the plasma membrane only.

Two possible sources of injury to the egg were considered. Insertion of the electrodes caused an injury seen as a lowered resistance and transmembrane potential that recovered in 3–5 min. In a few eggs, the resistance began at a high level, 4 megohms,
and fell over a 10-20 min period to about 1 megohm. This raises the possibility that the relative steady resistance before first division involved a stabilized form of injury. Also, a small mass of cytoplasm lumped around the shaft of the electrode appeared routinely and developed over a 10-30 min period. The membrane resistance often increased at the same time the reaction appeared, so a spontaneous creation of a leakage resistance probably did not occur. In any case, conclusions drawn here regarding new membranes are largely independent of a steady leak resistance due to injury.

**RESULTS**

Division in Solutions Permitting Normal Development

\(\frac{3}{4}R\) Ringer was established as the upper limit for normal development. Figs. 1 and 2 show the changes in electrical parameters in time for 2 of 10 eggs studied in either \(\frac{1}{2}R\), \(\frac{1}{4}\), or \(\frac{1}{2}\) Ringer.

A general result was that the capacitance of these eggs either remained constant or decreased slightly and steadily. The increase in geometrical surface area associated with the furrow was therefore not reflected by an increase in capacitance in eggs dividing normally.

The resistance typically decreased at the appearance of the first furrow, often followed by a recovery to greater than initial values. This was in turn followed by a fall in resistance at the second division.
potential before division was generally about \(-20\) mv. Paralleling the fall in resistance, the potential became increasingly negative inside, and then decreased, often to the level before division.

Fig. 3 illustrates a normal division of an impaled egg. Some eggs dividing normally showed very little change in resistance or potential at division; the egg in Fig. 3 was of this type.

**Failure of Division of Eggs in Full Ringer**

An unsuccessful attempt to divide in full Ringer was accompanied by greater changes in resistance and potential than were found for eggs dividing success-

![Figure 2. An egg in \(\frac{1}{2}\) Ringer. Diameter, 1600 \(\mu\); specific capacitance, 1.16 \(\mu\)F/cm\(^2\) (assuming a total of \(13.5 \times 10^{-8}\) F); specific resistance at 65th min, 74,000 ohm cm\(^2\).](image)

The positive shift in potential at the 5th min was caused by insertion of second electrode. A mechanical disturbance caused the positive shift at the 69th min.

fully in more dilute solutions. Substantial increases in capacitance were also found. Also, a marked sensitivity of the potential to the concentration of K in the bathing medium appeared during the attempt at furrowing.

Fig. 4 shows the results for one of five eggs placed in a low Cl Ringer. A distinct increase in capacitance was correlated with the appearance of the first and second furrows, as well as a decrease in resistance and increase in potential. These changes were comparable in magnitude to those found in a high Cl Ringer.

Sensitivity of the potential to K in the bathing medium is illustrated by eggs attempting to divide in Ringer containing either 0 or 20 mM K (see
Figure 3. An egg dividing in 3/4 Ringer. Upper left, before first division; upper right, the beginning of first division; tension lines radiate perpendicular to the plane of cleavage. This stage corresponds to the notation 1st on the figures showing electrical data. Comparable stages exist for the notation 2nd and 3rd. A small mass of cytoplasm has collected at the point of insertion of the microelectrodes. Lower left, end of 1st division; lower right, end of 2nd division.

Figs. 5 and 6). The egg shown in Fig. 5 reached $-69 \text{ mv}$ in 0 mM K at the third attempt at furrowing whereas the potential of the egg shown in Fig. 6 remained near $-32 \text{ mv}$ in 20 mM K. The microelectrodes were removed for a time from the egg shown in Fig. 6. Reinsertion gave comparable values of resistance and potential and revealed the continued increase in capacitance.
A gradual increase in resistance during the interkinetic stages is evident for eggs in full Ringer even while stable increases in capacitance were present. The changes in potential at division for three eggs studied in 122 mM K were reversed in polarity (see Fig. 7).

The total surface area of the egg increases during the development of a furrow, so it is reasonable to assume that an introduction of new plasma membrane onto the surface is the mechanism for the increase in capacitance.

If no increase could be observed under any circumstances during a single division cycle, it could be argued that the actual growth of membrane does not occur during normal furrowing. The apparent increase in area might then be accomplished by a simple unfolding of gross invaginations of the membrane. The increase in capacitance observed in full Ringer, in contrast to the absence of an increase in more dilute solutions, is evidence that new membrane is in fact introduced onto the surface during the growth of the furrow and not before or after. The absence of a capacitance increase for an egg in dilute Ringer can be accounted for by the presence of a high electrical resistance.
between the furrow membrane and the bathing medium due to a narrow gap between the blastomeres (see Appendix—Electrical characteristics of the furrow gap).

Two problems are considered in the Appendix. (a) How much furrow membrane capacitance can be detected in the case of a normal furrow? (b) What is a reasonable value for the specific resistance of new membrane within the furrow? The analysis shows that the maximum per cent increase in capacitance for a single furrow in $\frac{1}{2}$ Ringer is 3.3%, and therefore this might not be detected with the methods used. A value of 950 ohm cm$^2$ was computed for specific resistance of new membrane in the furrow. This is comparable to the value, 550 ohm cm$^2$, found for the "junctional membrane" between cells in the Triturus egg (Ito and Hori, 1966).

Visual observations of the surface of eggs with furrowing inhibited suggest that new membrane formed within the furrow is ejected out onto the surface of the egg and exposed directly to the bathing medium when the mechanical constrictions relax and the surface flattens out. An example of the inhibition
of furrowing is shown in Fig. 8 where the egg was bathed in \( \frac{1}{2} \) Ringer plus 150 mM sucrose beginning 48 min before the first division. A series of 16 eggs bathed in this medium gave electrical results with inhibition of cleavage that were similar to those in full Ringer. As shown in Fig. 8, the tension lines developed in these eggs and the furrow began to cut through the cytoplasm. The mechanical constrictions of the surface later relaxed and the surface evened out, leaving no evidence of a pair of membranes extending into the cytoplasm.

Under conditions which inhibit cleavage, it is also possible that new membrane might be produced but then reabsorbed, or not produced at all. Instances have been found, when furrowing was rapidly disrupted, in which no capacitance increase was observed. The changes in resistance and potential were also less than usual. The egg shown in Fig. 8 was of this type.

![Graph showing electrical activity](image-url)
The mechanical constrictions of the egg surface do not appear to be obligatorily related to the electrical changes. The fact that the surface constrictions begin shortly before the start of the electrical changes indicates that the electrical phenomena do not trigger the mechanical changes. Also, the large deformations of the surface shown in Fig. 8 and relatively small electrical changes indicate that mechanical constrictions and electrical changes are due to independent processes which may be dissociated.

Figure 7. An egg in 122 mM K. NaCl was replaced by KCl in full Ringer. The diameter was 1430 μ, specific capacitance, 1.24 μF/cm² before first division (assuming total of 8 × 10⁻⁸ F); specific resistance at the 10th min, 16,000 ohm cm².

Membrane Potential vs. Extracellular K Concentration

Transmembrane potential vs. concentration of K in the bathing medium is plotted in Fig. 9 for the eggs showing the maximum changes in electrical parameters yet found to be correlated with furrowing.

These results are characteristic of a membrane permeable mainly to K. The deviation from the theoretical slope of 59 mv per 10-fold change, especially at the low concentrations of K, indicates a small but significant contribution to the total conductance from other ions. The intersection of the line at 0 mv suggests that the intracellular concentration of ionized K is near 88 mM.
FIGURE 8. An egg in 1/2 Ringer plus 150 mM sucrose. Upper left, 15 min after initial penetration, at start of first division. Upper right, 30 min, still in first division. Lower left, 68 min, beginning of second division. The first furrow no longer extends into interior. Lower right, 107th min, beginning of third division attempt, the first and second furrows have relaxed. A lightly pigmented area marks site of the first furrow.

**The Zero Current Potential of New Membrane**

The preceding results show that a sensitivity of the membrane potential to K develops during the disruption of furrowing. The slow introduction of new membrane, assumed to cause the change, would be equivalent electrically to a slow, continuous addition in time of a conductance and a voltage element, zero current potential, in series with each other and in parallel with the existing membrane conductance and voltage element. The zero current potential
Figure 9. Transmembrane potentials of eggs after one to three division cycles in solutions of varying K concentrations. The line, drawn by eye, has a slope of 50 mV per 10-fold change in K concentration and intercepts 0 mV at 88 mM K. Dots and crosses, two eggs in 1/2 Ringer + 150 mM sucrose; open circles, the potential maxima for the data in Fig. 6; triangles, the potential maxima for the data in Fig. 7; open square, the maximum in potential at first division in Fig. 8. NaCl was substituted for by KCl in all solutions. Points on 1 mM K ordinates correspond to K-free solution.

Figure 10. An equivalent circuit of new membrane in parallel with preexisting membrane.

Of the new membrane can be estimated from the relation between change of potential and resistance soon after the start of the first furrow. Let \( V_m \) be the transmembrane potential, \( g_1 \) and \( g_2 \) the conductances, and \( E_1 \) and \( E \), the zero current potentials of two conducting networks in parallel (see Fig. 10). The total current through both is given by

\[
I = g_1(V_m - E_1) + g_2(V_m - E).
\]
We differentiate $I$ with respect to $g_2$, let $g_1$ be constant, and let $g_2$ approach zero. The lumped resistance of the two elements is defined as $R = 1/(g_1 + g_2)$. From the relation $dg_2 = -\left(\frac{dR}{R^2}\right)$, and since $I = 0$ at the resting potential, the following equation is obtained

$$E = V_m - R\left(\frac{dV_m}{dR}\right).$$

This equation allows the zero current potential, $E$, to be calculated from the membrane potential, the resistance, and the ratio of the rates of change of voltage and resistance at a point in time. The conductances and zero current potentials of the preexisting membrane and the new membrane already introduced are assumed to be constant. The conductance of the element being added in time is assumed to be linear in the range from $V_m$ to $E$, but other conductances need be linear only in the voltage range used for the measurements of resistance.

The mean value of $E$ for 14 eggs, including 11 in full Ringer and 3 in low Cl Ringer, was $-68.4 \pm 6.3$ mv (standard error of mean). The mean potential and resistance before furrowing were $-15.6 \pm 2.3$ mv and $1.08 \pm 0.18$ megohm. The mean values of potential and resistance at the peak of the potential change were $-36.6 \pm 2.4$ mv and $0.42 \pm 0.07$ megohm.

Considerable variation existed between eggs. Some eggs in full Ringer had stable furrows for a time and did not show inhibition of furrowing until the fourth or fifth division. Generally, the more rapid the onset of inhibition, the greater the change in potential at first division.

The computed values of $E$ were in every case above the final value of the voltage maximum, so there seems to be no reason to believe that $E$ itself increases in time. Also, except for the few cases in which the resistance before division was increasing substantially, the values of $E$ are low enough to be accounted for by a simple diffusion potential, with the membrane most permeable to K.

Two observations indicate that the existing conductances do vary somewhat. First, the resistance of the preexisting membrane of many eggs tended to increase gradually before the first sign of furrowing (see Fig. 4). If the pre-existing membrane were to increase in resistance at the same time new membrane appeared, high apparent values of $E$ would result. The phenomenon was also found in seven of eight cells that were exposed to a sperm suspension but showed no sign of furrowing. In these eggs the resistance increased gradually over a 30 min period beginning about the time when division would normally have occurred. The resistance stabilized at two to four times the initial level, and no change in potential accompanied the increase.

A second change in conductance involved an increase in resistance and loss of K selectivity of the new membrane after it was formed. The observations
arguing for the spontaneous alteration in properties are: the fall in potential from a maximum, a delayed increase in resistance, and a stabilized increase in capacitance after an attempted division. The stimulus for the change in characteristics may be that the new membrane is exposed to the external medium, an alien environment, rather than isolated as part of the border of a narrow furrow gap. Also, the actual transmembrane potential may be 40 or 50 mv more positive than the zero current potential of the new membrane, and the steady depolarization might affect the conductance properties. This steady loss of K selectivity of the new membrane already formed would yield low values for $E$.

These values for the zero current potential constitute evidence that the new membrane is selectively permeable to K within 2-3 min, if not immediately, after its appearance.

_Potentials in Multicellular Embryos_

It was of interest to study the membrane potentials of cells in more advanced embryos in which it is certain that the cell membranes are formed _de novo_ during development.

Two microelectrodes of 25 megohms resistance were used to record potentials and pass currents. Embryos used for these studies were allowed to develop in 1/10 Ringer until they were used.

24 hr old embryos were placed in 1/2 Ringer. In one embryo, a potential of -50 mv, presumably intracellular, was recorded for 20 min. Another of -49 mv was recorded for 40 min. At the same time, the second electrode was in a region at +17 mv. Current passed through the electrode in the positive region resulted in a voltage change recorded by the electrode in the negative region corresponding to a resistance of 0.35 megohm. Potentials of -59, -63, and +10 mv were recorded in another embryo, and the resistance was 0.28 megohm. Other embryos gave similar results.

Similar experiments were performed on 3- and 4-day embryos placed in full Ringer. The electrodes were pointed at the belly of the embryo where the cells are large. Potentials of -52, -48, -56, -57, -50, -54, and +30 mv were found in 3-day embryos. The resistance was 0.18 megohm. Potentials of -65, -59, -56, -55, and +29 mv were found in 4-day embryos and the resistance was 0.11 megohm. Often when probing with an electrode the tip went directly from a positive region to a negative region. Deterioration of a cell often resulted in a negative potential drifting to a positive potential.

A simple interpretation of these results is that the surface of the embryo behaves as an epithelial layer in which the magnitudes of the transmembrane potential differences are not equal at the two surfaces. This arrangement of two membranes in series could generate a positive potential extracellularly within the embryo and a negative potential within the cells.
The difference between the positive and negative potential, -70 to -80 mv, is probably across membrane formed de novo during development. This is to be compared with -68 mv computed for $E$, the zero current potential of new membrane; also, the range of -70 to -80 mv falls in Fig. 9 at 2-3 mm K, suggesting that the intercellular concentration may be regulated at that level in embryos.

**DISCUSSION**

A simple interpretation of the results is that new plasma membrane is formed only within the division furrow and is permeable to K immediately upon its appearance. This model is supported in several ways. The sensitivity of the membrane potential to the concentration of K in the bathing medium seen after furrowing is inhibited is evidence that the new membrane is permeable mainly to K. The determination of the zero current potential of the increasing conductance element at the first division gives evidence that the new membrane is permeable to K immediately, or within 2-3 min, after its formation. The analysis of the electrical characteristics of the geometry of the furrow indicates that a narrow furrow gap could limit current flow through the gap membrane and lead to relatively small changes in resistance, potential, and capacitance for eggs dividing normally. Measurements of transmembrane potentials of cells in more advanced embryos also suggest that membrane formed during early development has permeability characteristics which could lead to a large negative intracellular potential.

Finally, the fact that the capacitance does increase correlated with the aborted furrowing indicates that new membrane is produced during the furrowing process and not before or after. Failure to find the expected capacitance increase in normal division suggests that the new membrane is electrically isolated within the furrow.

The capacitance data correlate well with the electron micrographs of developing cleavage planes in most other cells. Studies of the cleavage planes of mammalian cells (Buck and Tisdale, 1962), clam eggs (Humphreys, 1964), and onion root tip cells (Porter and Machado, 1960; Frey-Wyssling, Lopez-Saez, and Muhlethaler, 1964) show a common scheme for cytokinesis. Vesicles line up within the cytoplasm along the equatorial plane and coalesce into two continuous sheets, with division accomplished by the peeling apart of two preformed membranes. Such a mechanism could yield the electrical results found here for the frog egg.

Alternate interpretations of the results are possible. A modification of any patch of plasma membrane could lead to a lower resistance and an increase in permeability to K; and, the capacitance could increase by means of a change in the dielectric constant or thickness of the membrane. Another scheme might involve the addition of new membrane dispersively throughout
the preexisting membrane with the capacity held constant by a migration of membrane into the narrow furrow. While other logically consistent schemes can be given, each would require more ad hoc processes than the model proposed here which integrates the observed phenomena in the simplest fashion I can think of. In any case, further experimental analysis will follow paths suggested by the model.

Direct evidence for an increase in conductance of the membrane in the furrow region is not provided here; however, three studies of electrical correlates of division in other cells support the idea. Hyde (1904) found an extracellular positivity on the surface of the Fundulus egg at the region where cleavage furrows form and at the time they appear. Lund (1947) found a positivity on the surface of a thread of alga at the time and site of formation of the cleavage plane between cells. Also, Romanoff and Cottrell (1939) found an extracellular positivity on the surface of the blastodisc of the developing hen's egg where active proliferation of cleavage furrows takes place.

In each of these cases, the experiments were performed so that the extracellular pathway for current flow away from the positive region would constitute a high resistance. The cells were suspended in air so that a thin layer of conducting fluid covered the surface. If the cytoplasm of these cells were an isopotential region and if the membrane in the furrow were to have a high negative zero current potential compared to that of the surrounding membrane, then the high extracellular resistance would favor the development of positive extracellular potentials in the region of the furrow due to the flow of local currents. The Fundulus egg is known to have a low membrane potential, about 0 mv, after fertilization (Kao, 1956) so the extraceUular positivity might reasonably be present at division.

Changes in transmembrane potentials similar to these in the frog egg cannot be expected from all dividing cells, particularly if the preexisting membrane were to have a high transmembrane potential at the outset. For example, a constant potential of -50 mv has been found during division for the egg of the starfish Asterias (Ashman, Kanno, and Loewenstein, 1964).

Other studies of amphibian eggs have confirmed the general result that the resistance falls and the potential becomes more negative at division. Maeno (1959) found comparable high resistances and low membrane potentials after fertilization in the Bufo egg, but he did not record during division. Ito and Hori (1966) reported a fall in resistance at first division for the Triturus egg in 0.1 Holtfreter's solution. Final values were comparable to those reported here. The mean values of membrane potential, however, rose steadily from -6 mv at the one-cell stage to -54 mv at the late morula stage. Morrill and Watson (1966) observed a +40 mv potential for Rana pipiens eggs in 1/16 Ringer after fertilization followed by a slow reversal during the first division to a "relatively constant" level between -50 and -60 mv. They
did not measure resistance or capacitance. The most apparent difference in results between workers is in the rate and magnitude of change in potential at division. This variation could be due to differences in bathing solution, the properties of the preexisting membrane, or to the extent to which current was able to flow out of the furrow gaps for the particular eggs studied.

The results suggest that a phase of mechanical constriction precedes the period of new membrane production. The finding that no electrical changes accompany the initial constriction and depression of the surface suggests that the preexisting membrane stretches passively and extends slightly into the surface of the egg before new membrane is introduced. This concept of an initial phase of passive stretching followed by a phase of new membrane production parallels that developed by Dan (1954) in his investigations of de novo production of membrane in invertebrate eggs. In the small invertebrate egg, the mechanical constriction appears to proceed until the egg is separated by a small stalk, with the active production of membrane area occurring at the end of the division process. It would seem that the phase of new membrane production is more apparent in the amphibian egg whereas the initial mechanical constrictions are more apparent in the invertebrate egg.

The electrical properties of the furrow gap were analyzed on the basis of a 200 A gap between the furrow membranes. With minor additional assumptions, the data are also consistent with a model in which the furrow membranes normally fuse to form a zonula occludens (Farquhar and Palade, 1963) or nexus (Dewey and Barr, 1964) at a part or all of the outer rim of the furrow. The large membrane resistances found for embryos at 24 hr (0.35 megohm), where the junctional contact between cell surfaces has increased enormously compared to a single furrow, suggest that such structures do appear at some time during the early cleavage cycles. Eggs displaying minimal electrical effects during first division may form a nexus immediately upon formation of the furrow. An early closure of the gaps between cells would be of value to the frog embryo developing in freshwater, as this would limit the area available for osmotic influx of water.

The conductance properties of the new membrane are not stable since the resistance increased and potential decreased after the capacitance increased and remained steady. These changes may be due to effects of steady outward current through the membrane, or due to the exposure of new membranes to the bathing medium. Also the possibility that these effects are related in some way to cytoplasmic events during cytokinesis cannot be rejected.

The "normal" membrane potential and resistance of an individual egg dividing successfully may be a result of several influences identified here: the resistance and potential of preexisting membrane; extent and timing of closure of furrow gaps; and possible alteration of properties of new membrane.
at the outer rim of the furrow. These processes may be reflected in the quantitative differences between studies on amphibian eggs.

Little is known, in general, about the mode of production and turnover of surface plasma membrane. Analysis of new membrane production in the frog egg may yield further information about these processes.

APPENDIX

Electrical Characteristics of the Furrow Gap

The difference in results between eggs dividing normally and those with furrowing inhibited can be accounted for by the existence of a narrow gap between the blastomeres. A sufficiently high electrical resistance of the fluid in a narrow gap could prevent the passage of capacitative and resistive current through membrane located deep in the furrow. To examine this idea, two questions were asked: how much of the furrow membrane capacitance can be detected with the method used; and, what are reasonable values for the specific resistance of the furrow membrane?

Electron micrographs of the outer edge of a furrow were prepared to obtain an estimate of the width of the furrow gap. As shown in Fig. 11 a, the spherical outer membranes turn in at the edge of the egg and converge at a well-defined point. The furrow gap membranes extend closely apposed into the interior of the egg. Typically, the width of the gap at the point of contact of the outer membranes was about 200 Å (Fig. 11 b). Beyond 10 μ into the furrow the gap membranes separated to 500 Å or more, but these regions were not as well-fixed.

The electrical characteristics of the whole egg can be considered as two conducting pathways in parallel (see Fig. 12). One path is through the outer, spherical membrane and can be described by a simple resistance and capacitance in parallel. The second path is through the furrow gap membranes and out through the edge of the furrow. The geometry of this second path consists of two radially symmetrical disc-shaped membranes separated by the width of the furrow gap.

An equation describing the conducting properties of the furrow is derived as follows. We define: d(cm), the gap width; x(cm), the radial distance; $R_g$ (ohm cm$^{-2}$), the specific resistance of the furrow membrane; $C_g$ (μF/cm$^2$), the specific capacitance of the furrow membrane; r (ohm cm), the resistivity of the gap fluid; and r (cm), the radius of the egg. Also, we let $I_g(x, t)$ (amp/cm$^2$) be the specific membrane current through the furrow membrane, defined positive in the direction from inside the egg into the furrow gap; $E$ be the potential difference across the gap membrane at which $I_g = 0$; and, $V_m$ be the potential of the cytoplasm, with the external medium defined at zero potential. $I(x, t)$ denotes the current within the gap directed radially outward. The blastomeres were electrically coupled so that $V_m$ is assumed equal everywhere within the cytoplasm.

The potential of the fluid in the gap relative to the external medium is denoted by $V_g(x, t)$, where $V_g(r, t) = 0$. The “electrotonic potential” $V(x, t)$, across the gap membrane is defined by:

$$ V(x, t) = -V_g(x, t) - E + V_m. $$
FIGURE 11. Eggs at the beginning of second division were fixed 30 min in 2% osmium tetroxide buffered to pH 7.4 with Tris hydrochloride, dehydrated in an ethanol series, and transferred to an Epon embedding medium via propylene oxide. Sections were cut perpendicular to the completed first cleavage furrow at the outer edge. General features of a cleavage furrow are shown in Fig. 11 a. Bar, 5 μ. The outer, spherical membranes (out of picture) converge gradually and contact at a well-defined point and then extend as "furrow membranes" into the interior of the egg. Fig. 11 b shows the initial region of contact of the furrow membranes of another egg. Bar, 0.2 μ. The distance between the centers of the dense lines is 190 Å. The opening to the outside medium is out of the picture at the upper left corner. The closely apposed membranes merge briefly with a vesicular structure and then extend into the interior.
The change in electrotonic potential, $V$, with radial distance is given by equation 1. Since $\partial V/\partial x = -\partial V_0/\partial x$,

$$\frac{\partial V}{\partial x} = \frac{I_0}{(2\pi d)}. \quad (1)$$

The change in gap current, $I$, due to the gap membrane current entering or leaving, is described by equation 2. Note that two gap membranes contribute current.

$$\frac{\partial I}{\partial x} = I_0 4\pi x. \quad (2)$$

Figure 12. An equivalent circuit of the furrow membranes and outer spherical membrane. The close apposition of the disc-shaped furrow membranes results in a cable-like circuit, here illustrated at the outer edge of the furrow for the membrane of one blastomere. The labels $C_{\phi}$, $R_\phi$, and $\rho$, are related to values of ring-shaped circuit elements.

The membrane current due to the resistive and capacitative components is given by equation 3.

$$I_\phi = \frac{V}{R_\phi} + C_\phi \frac{\partial V}{\partial t}. \quad (3)$$

Equation 1 is differentiated with respect to $x$ and equations 1, 2, and 3 are substituted into the result to obtain equation 4. Two new quantities are defined: the length constant, $\lambda = (R_\phi / 2\rho)^{1/2}$; and the time constant, $\tau = R_\phi C_\phi$.

$$\frac{\partial^2 V}{\partial x^2} + \frac{1}{x} \frac{\partial V}{\partial x} - \frac{V}{\lambda^2} = \frac{\tau}{\lambda^2} \frac{\partial V}{\partial t}. \quad (4)$$

Letting $X = x/\lambda$ and $T = t/\tau$, equation 4 is expressed in dimensionless units by equation 5.

$$\frac{\partial^2 V}{\partial X^2} + \frac{1}{X} \frac{\partial V}{\partial X} - V = \frac{\partial V}{\partial T}. \quad (5)$$
Most of the useful information about equation 5 can be obtained from its solution at the steady state when $\frac{dV}{dt} = 0$. The solution for a steady applied current is a bessel function with an imaginary argument, $J_0(iX)$ (Jahnke and Emde, 1945). $J_0(iX)$ approximates an exponential function within small intervals and at large values of the argument. If we assume that the length constant is small compared to the radius (which will be justified below), the electrotonic potential can be described by $V = V(r) \exp(-x/\lambda)$ where $x$ is defined positive into the furrow and $x = 0$ at the rim of the furrow gap. Using this approximation for $V$, the input resistance of the furrow gap, defined as $V(r)/I(r)$, can be shown from equation 1 to be $(R_0/d)^{1/2}/2\pi r$.

We will assume a typical egg radius, $r$, of 750 $\mu$; a $\rho$ of 150 ohm cm, about that of $1/2$ Ringer’s; and a gap width of 200 A. The fall in resistance at the appearance of the first furrow in Figs. 1 and 2 was equivalent to the addition of a 0.3–0.5 megohm resistance in parallel with the preexisting resistance. Assuming 0.4 megohm as the input resistance of the furrow, $R_0$ is found to be 950 ohm cm$^2$ and the length constant $\lambda$, 25 $\mu$.

The error in assuming an exponential form for $V$ was evaluated by direct computation of $J_0(iX)$ and $d(J_0(iX))/dX$ using an IBM 1620 computer. Values of $J_0(iX)$ were normalized at $X$ ranging from 1.0 to 20.0 and compared with values from the exponential approximation. The results showed that less than a 10% error is involved for the assumptions employed. With values of $X$ greater than 10, the fraction of error in computing $R_0$ is approximately $\lambda/r$. The assumptions of a constant $d$, $R_0$, and $\rho$ are therefore more important and probably limit the accuracy of $R_0$ to within a factor of two.

One method of obtaining an upper limit for the measurable furrow membrane capacitance is to assume that the furrow membrane capacitance charges instantaneously to the steady state as the membrane potential, $V_m$, is changed. The total capacitative charge, $q$, on the furrow membrane, given by

$$q = \int_0^{r/\lambda} 4\pi r XC_m X^2 V(X) dX,$$

is divided by the electrotonic potential at $X = r/\lambda$ to compute an effective membrane capacitance. This value is divided by the capacitance of the outer spherical membrane, $4\pi r^2 C_m$, and multiplied by 100 to obtain the maximum per cent increase in capacitance measurable due to the appearance of the first furrow. The specific capacitance of the outer spherical membrane is assumed to be equal to that of the furrow membrane, and $V$ is assumed to be described by the function, $V(X) = V(r)AJ_0(iX)$. $V(r)$ is the electrotonic potential at $x = r$ and $A$ is a constant such that $AJ_0(ir/\lambda) = 1$.

The resulting function, denoted $\%C$, is:

$$\%C = 100 \left(\frac{r}{\lambda}\right)^2 \int_0^{r/\lambda} AJ_0(iX) X dX$$

The individual terms in $J_0(iX)$ were multiplied by $X$, integrated, and divided by
\((r/\lambda)^2\). The resulting series is equivalent to the function,

\[
\% C = \frac{100\lambda A}{r} \frac{\partial}{\partial X} [J_0(iX)]X_{\sigma/\lambda},
\]

and values for \(\% C\) were obtained from the results of the previous computation.

The per cent increase in capacitance was found to equal 9.5% when \(X = 10\) and converge to the ratio \(\lambda/r\) at larger values of \(X\). Thus, with values of 25 and 750 \(\mu\) for \(\lambda\) and \(r\), the maximum detectable per cent increase in capacitance is 3.3%. This value is at the limit of the accuracy of the capacitance measurement so it is reasonable that no capacitance increase was found for eggs dividing normally. The slight upward drift of capacitance in Fig. 2 for the egg in \(\frac{1}{2}\) Ringer may be accounted for by a possible 6.6% increase by the end of the second division. The specific resistance of the fluid in the furrow gap is likely to be greater for the eggs in \(\frac{1}{2}\) Ringer so that the measurable capacitance would be proportionately smaller.

A 50% increase in capacitance is possible if \(R_o\) were infinite and all the gap membrane eventually became charged. However, the capacitance was determined from the initial rate of change of voltage during the first 2 msec of the test pulse; and, it can be shown that a significant time delay, due to the cable properties of the furrow, would exist before interior portions of the furrow begin to charge. The finding of no capacitance increase therefore does not by itself argue for a low resistance gap membrane.

In summary, the determinations of the furrow gap width and electrical properties of eggs dividing normally are consistent with the postulate that the new membrane has a low resistance of 950 ohm cm\(^2\) compared with that of the preexisting membrane, 25–100 kohm cm\(^2\). Current flow would be limited to a 25 \(\mu\) deep segment at the outer edge of the furrow.

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REFERENCES


D. J. Woodward  Electrical Signs of New Membrane Production


