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On the Mechanism of Electrical Coupling between Cells of Early *Xenopus* Embryos

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Summary. The mechanism of electrical coupling between cells of early *Xenopus* embryos has been studied by examination of the nonjunctional membrane resistances and capacitances as a function of cleavage stage, the junctional and nonjunctional membrane resistances as functions of time during the first cleavage, and the electrical properties of the primitive blastocoel. The changes in membrane resistances and capacitances during the first two cleavages may be completely explained by the addition of new membrane, identical in specific resistance and capacitance to the original membrane, at a constant rate to furrows which are electrically connected to the perivitelline space. Microelectrode recording from the primitive blastocoel indicates that there is no electrical difference detectable between it and the perivitelline space. These results are discussed in the context of current theories of the mechanism of intercellular electrotonic coupling.

The flow of electric current between animal cells via relatively low resistance pathways has been demonstrated in a number of adult and embryonic tissues (*see* reviews by Loewenstein, 1966, 1968; Furshpan & Potter, 1968; Bennett, 1973). It is now recognized that in many instances the low resistance pathways may be morphologically identified as gap junctions between the cells (Payton, Bennett & Pappas, 1959; Gilula, Reeves & Steinbach, 1972; Azarnia, Larsen & Loewenstein, 1974), characterized by the presence of a 20–30 Å intercellular space in which subunits may be observed after lanthanum infiltration and freeze fracturing (McNutt & Weinstein, 1973).

In previous reports we have described the electrical resistance and capacitance properties of early *Xenopus* embryos up to the eight-cell stage (DiCaprio, French & Sanders, 1974, 1975). In each stage we discovered that all of the cells in an embryo were identically connected to all of the other cells and that cytoplasmic processes which might mediate such connections could be demonstrated by light and scanning electron-

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microscopy. In view of the known morphology of the dividing early *Xenopus* embryo (Sanders & Zalik, 1972; Singal & Sanders, 1974), which indicates that the first cleavage furrow is open to the external medium and that intercellular contacts having some of the attributes of gap junctions are present between the cells in these early stages, we have suggested that electrical current flows directly between cells of early *Xenopus* embryos via gap junctions. This proposal has also been made for the embryos of *Triturus* (Ito & Hori, 1966; Ito & Loewenstein, 1969).

Other authors have suggested an alternative explanation for electrical coupling in early amphibian embryos. They have postulated that the new membrane forming the cleavage furrows has a lower resistance and a higher permeability to potassium ions than the existing membrane and that the perimeter of the primitive blastocoel is physically sealed, from the first cleavage onward, so that the blastocoel is electrically isolated from the external medium. Electrical connections between cells are then supposed to be mediated by the passage of electric current through the blastocoel (Woodward, 1968; Slack & Warner, 1973; deLaat & Bluemink, 1974). It is clearly important to distinguish between these two alternative mechanisms in view of the possible importance of intercellular coupling to embryogenesis. If the second mechanism is correct then electrical coupling in amphibian embryos is qualitatively different from that found in other tissues.

In order to further clarify this situation, we have now measured the junctional and nonjunctional membrane resistances during the first cleavage and the membrane capacitance before the first cleavage. We have also made direct electrical measurements on the primitive blastocoel at the end of the third cleavage. These measurements, which were not previously available, are discussed in terms of the two alternative mechanisms of intercellular coupling.

Materials and Methods

Embryos of *Xenopus laevis* were obtained by injection of adult animals with Antuitrin-S (Parke Davis & Co.). Females received 1 000 IU and males 500 IU of the hormone subcutaneously. Embryos were kept in Steinberg's solution (Hamburger, 1960) throughout the experiments and dejellied in a solution of papain-cysteine hydrochloride for approximately 2 min at room temperature (Dawid, 1965). After treatment with the enzyme mixture the embryos were washed in fresh saline a minimum of seven times in order to remove all traces of the enzyme. The vitelline membrane was left intact in all experiments.

Embryos were placed on a layer of paraffin wax in a shallow glass dish filled with Steinberg's solution. Several embryos at the same stage of development were placed together in the dish but only one was used for electrical measurements. Normal development of the experi-

mental embryo was monitored by comparing its external morphology to the control embryos. Glass microelectrodes filled with 3 M KCl and having resistances of 3–5 MΩ were used for the injection of electric current and the measurement of intracellular voltages. The saline bathing medium was returned to electrical ground via a 3 M KCl/Agar bridge and an Ag/AgCl junction. The recording electrodes were connected via Ag/AgCl junctions to bridge electrometers (W. P. Instruments) having resistance and capacitance compensation. All stimulating currents passed directly through a 10^8 Ω resistor and an Ag/AgCl junction to a current injecting electrode. This arrangement allowed all network analysis to be performed in terms of the injection voltage and also allowed the resistance of the injecting electrode to be ignored.

The following behavior was considered to indicate abnormal development of the experimental embryo: (1) reversal of the cleavage furrow after electrode insertions; (2) displacement of the electrodes by the developing furrow and consequent unsealing; (3) spurious furrows appearing around the electrodes; (4) abnormal development following removal of the electrodes. For the last criteria each embryo was observed until it reached the late blastula stage.

The membrane time constant of early *Xenopus* embryos is sufficiently small that an oscillating current below about 5 Hz may be considered to pass entirely through the membrane resistances and not through any membrane capacitances (DiCaprio *et al.*, 1974). In order to make voltage measurements independent of the cell membrane potentials we injected a constant sinusoidal current of 0.5 Hz into the embryo. The injection voltage and the intracellular voltage were then recorded at the time of the experiment using a paper chart recorder and their amplitudes were measured as peak-to-peak voltage fluctuations. With this technique the modulations in intracellular voltages due to the resistive properties of the membranes were observed with the sinusoidal traces free to change their mean positions with fluctuations in the membrane potentials or electrode tip potentials.

Direct measurement of the voltage in the primitive blastocoel was accomplished by use of a calibrated hydraulic micromanipulator (David Kopf Instruments). This instrument allowed movement of a microelectrode in one dimension with measurement of relative position to a resolution of 1 micron.

The resistance and capacitance of the single cell membrane was calculated from the frequency response function between the injecting voltage and the observed intracellular voltage during wide bandwidth random stimulation. The analytical technique has been described in detail before (DiCaprio *et al.*, 1974). A white noise source, obtained from a noise generator with a flat power bandwidth in the region 1–400 Hz (French, 1974) was band-limited to 50 Hz by a nine-pole active filter and then fed to the injecting electrode. The continuous input and output signals were sampled by an analog-to-digital converter at 10 msec intervals and processed on-line by a PDP-11/40 computer using software similar to that described previously (French, 1973). The frequency response and coherence functions showed the expected form for a first-order linear system with a low level of extraneous noise. The membrane resistance was calculated from the low frequency asymptote of the gain curve. The time constant of the membrane was obtained from the corner frequency of the gain and phase curves and the membrane capacitance was calculated from the membrane time constant and the membrane resistance.

Results

The Resistance and Capacitance of the Single Cell

Experiments were performed to determine the membrane resistance (26 experiments) and capacitance (15 experiments) of fertilized uncleaved

embryos. The resulting values for resistance and capacitance were: $0.96 \pm 0.26 \times 10^6\ \Omega$ and $0.0428 \pm 0.0053\ \mu F$ respectively (mean $\pm$ standard deviation). The result for membrane resistance at this stage agrees well with that of deLaat and Bluemink (1974) of $0.852 \times 10^6\ \Omega$.

The early *Xenopus* embryo may be approximately modelled as a sphere of constant diameter where the first cleavage plane divides the embryo into two identical hemispheres. The area of new membrane which gives rise to the cleavage plane is therefore disc-shaped. A description of the deviations from this model and a demonstration that the cleavage furrows are formed from new membrane is given by Bluemink and deLaat (1973). If the radius of the sphere is r, then the area of the surface membrane at the single-cell stage is $4\pi r^2$, while the area of each cell surface membrane at the two-cell stage has one hemispherical portion, of area $2\pi r^2$, and a new membrane portion of circular form and area πr^2, giving a total membrane area of $3\pi r^2$. Continuation of this description to the second cleavage gives four cells having pre-existing membrane area of πr^2 and a new membrane of area πr^2, for a total membrane area of $2\pi r^2$.

In previous studies we have used membrane areas based on the above models to calculate the specific membrane resistances and capacitances, but both Bluemink and deLaat (1973) and Singal and Sanders (1974) have shown that the outer membrane surfaces of these embryos are irregular when examined by transmission and scanning electronmicroscopy. It has also been demonstrated in both studies that the deeper membranes of the furrows are much smoother in appearance than the outer membrane surfaces. Bluemink and deLaat (1973), on the basis of linear folding measurements, have estimated that the actual surface area of the outer membranes is about 70% higher than would be predicted by the simple geometrical models.

If the above figure of 70% increase in area for the outer membranes is used together with the assumption that the membranes in the cleavage furrow are in electrical contact with the external bathing solution (i.e., an open furrow model), it is possible to use the measured resistance and capacitance of the single cell to predict the nonjunctional cell membrane resistances and capacitances at later stages. Table 1 shows such predictions compared to the experimentally determined values which we have described previously (DiCaprio *et al.*, 1974). For both resistance and capacitance there is agreement between the predicted and measured values if a range of one standard deviation is allowed around each figure. Since both the resistance and the capacitance predictions are higher than the experimental measurements, the overall agreement would not be improved

Table 1. Predicted and measured cell membrane resistance and capacitance of *Xenopus* embryos during the first two cleavages[a]

Stage	Measured Resistance	Measured Capacitance	Predicted Resistance	Predicted Capacitance
1-cell	$0.96 \pm 0.26 \times 10^6\ \Omega$	$0.0428 \pm 0.0054\ \mu F$	–	–
2-cell	$1.05 \pm 0.16 \times 10^6\ \Omega$	$0.0239 \pm 0.0035\ \mu F$	$1.48 \times 10^6\ \Omega$	$0.0276\ \mu F$
4-cell	$1.80 \pm 0.20 \times 10^6\ \Omega$	$0.0106 \pm 0.0047\ \mu F$	$2.41 \times 10^6\ \Omega$	$0.0169\ \mu F$

[a] The measured values for the uncleaved egg are presented here for the first time and the predicted values for the next stages are based upon the uncleaved egg measurements and open furrow models with rough outside membranes and smooth furrow membranes. The measured values for the two- and four-cell stages are those reported by DiCaprio *et al.* (1974). *(See text.)*

by changing the amount of surface folding from the 70% estimate. The predictions and results of Table 1 indicate that the resistances and capacitances of the cell membranes during the first two cleavages can be adequately explained by a model of development in which the new membranes have the same electrical properties as the original membranes and the cleavage furrows are electrically continuous with the perivitelline space and external bathing solution.

Resistance Changes During the First Cleavage

Figure 1 illustrates the experimental approach that was used to observe the membrane resistances as functions of time. The single cell may be considered to consist of two parallel identical resistances R_m, each being the resistance of a hemispherical area of surface membrane, so that the resistance from inside the cell to ground is given by $R_m/2$. The two-cell stage consists of two complete cell membrane resistances R_m and a junctional resistance R_j which links the two cells. This description of the membrane resistances allows the embryo to be modelled as a single electrical network containing three resistive elements which are functions of time: two identical nonjunctional resistances $R_m(t)$ and the junctional resistance $R_j(t)$. Since there is no evidence for asymmetric development at this stage we assume that the two nonjunctional resistances $R_m(t)$ are identical. If electric current is now injected into one side of the cleaving embryo, while the voltages in the two halves are observed, it is possible to calculate the values of the resistances at any point in time by electronic circuit theory.

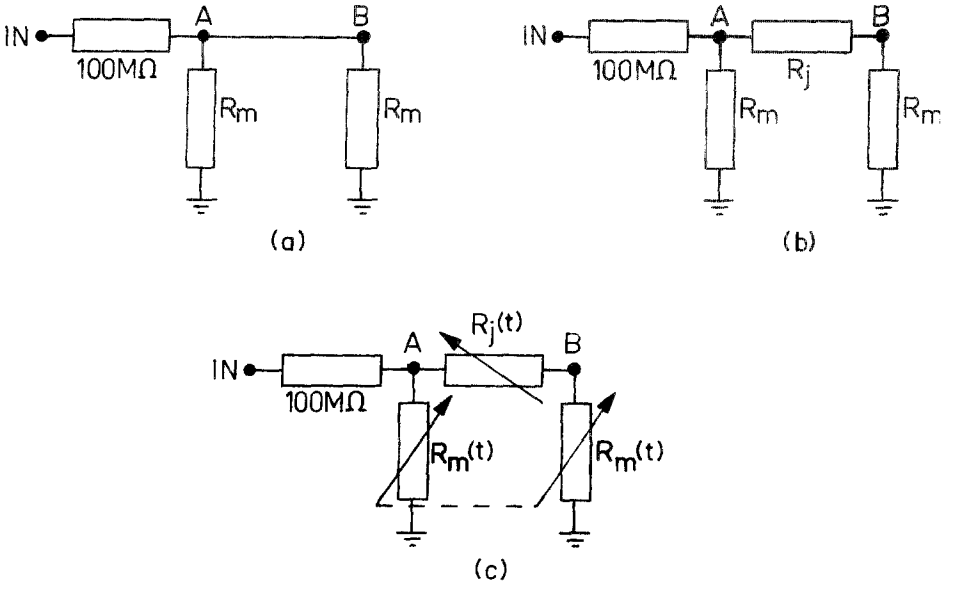

Fig. 1. Electrical models of the membrane resistances during the first cleavage of *Xenopus*. In each model the stimulating current was injected through node IN via the 100 MΩ resistance to node *A*. Recording microelectrodes were placed at nodes *A* and *B*. At the single-cell stage *(a)* the system has two hemispherical membrane areas, each of resistance R_m, connected in parallel so that nodes *A* and *B* are identical and represent the cell interior. At the two-cell stage *(b)* the two nonjunctional cell membrane resistances R_m are joined by a junctional resistance R_j and the nodes *A* and *B* represent the interiors of the two cells. The time-varying model *(c)* has two identical resistances $R_m(t)$ and a junctional resistance $R_j(t)$. The time-varying model may be applied throughout the cleavage process, with *(a)* and *(b)* representing the extreme variations

Using the circuit of Fig. 1*c* let the voltages at the input, cell *A* and cell *B*, be V_i, V_a and V_b, respectively, at some fixed time. Then let the total input resistance from cell *A* to electrical ground be R_i, where:

$$R_i = R_m(R_m + R_j)/(2R_m + R_j) \tag{1}$$

By Ohms law the voltage in cell *A* will be related to the input voltage by:

$$V_a = V_i R_i/(R_i + 10^8) \tag{2}$$

while the voltage in cell *B* is related to the voltage in cell *A* by:

$$V_b = V_a R_m/(R_j + R_m) \tag{3}$$

Rearrangement of Eq. 3 gives:

$$R_m = R_j V_b/(V_a - V_b) \tag{4}$$

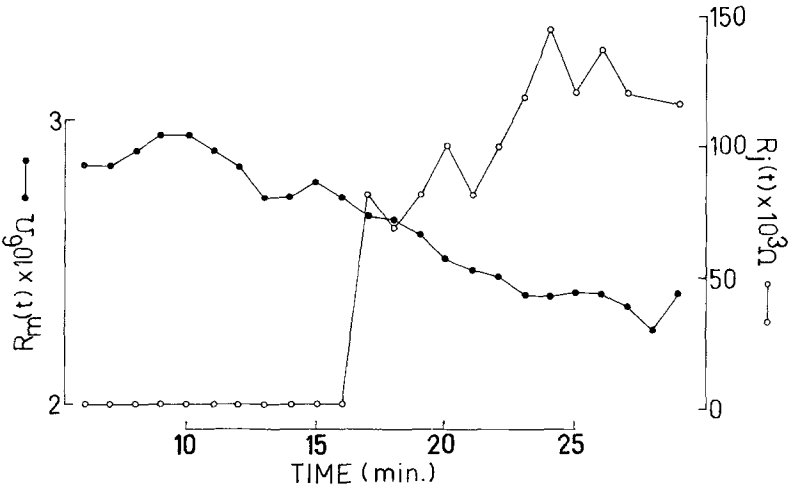

Fig. 2. The values of $R_m(t)$ and $R_j(t)$ from Fig. 1 during the first cleavage of an embryo. Time was measured from the instant at which all three microelectrodes were inserted into the embryo and measurements were taken at 1 min intervals thereafter. The second cleavage was observed to begin during the eighteenth minute and it was estimated that the first cleavage was complete at the twenty-sixth minute

Substitution of 1 and 4 into 2 gives:

$$R_j = 10^8(V_a^2 - V_b^2)/V_b(V_i - V_a) \tag{5}$$

R_j may therefore be computed from Eq. 5 with knowledge of V_i, V_a and V_b. R_m can then be obtained from Eq. 4 using the computed value of R_j.

Fig. 2 illustrates the results of such an experiment. The values of $R_m(t)$ and $R_j(t)$ are plotted versus the same time axis. Time was measured in minutes from the instant at which all three electrodes, one injecting current and two recording voltages, were inserted into the embryo. Immediately after insertion there was a large change in apparent membrane resistance as the result of a sealing process around the electrodes (DiCaprio *et al.*, 1974), and this process lasted about 6 min. The resistance values are therefore plotted from the sixth minute on and the slight initial rise in $R_m(t)$ is due to the termination of the sealing process. During the first cleavage illustrated in Fig. 2 the nonjunctional membrane resistance $R_m(t)$ decreased steadily, while the junctional resistance $R_j(t)$ was very low at the single-cell stage and did not rise until the seventeenth minute, when it rose sharply to more than 50% of its final value.

Staging of the embryos during these experiments was based upon the external morphology and upon previous experience of the electrical behavior of the embryos (DiCaprio *et al.*, 1974). The embryo used for

the experiment of Fig. 2 was at the far advanced groove stage (Singal & Sanders, 1974) at the time of electrode insertion. The normal time course of development would therefore place the start of the second cleavage at approximately 18 min, and completion of the first cleavage at approximately 26 min. The start of the second cleavage was confirmed by the appearance of the pigmented stripe during the eighteenth minute.

The simplest possible model of membrane modification during the cleavage is that the membrane of the cleavage furrow is produced by addition of new membrane, having identical properties to the existing membrane, at a constant rate with time. If the cleavage furrow does not provide a significant resistance to the flow of electric current, compared to the membrane resistances, all of the cell surfaces can be considered to be in direct contact with electrical ground at all times. This model predicts that the area of membrane through which current can flow to ground is increasing linearly with time and, since membrane resistance is inversely proportional to membrane area, the reciprocal of the membrane resistance should vary linearly with time. Fig. 3 illustrates the results for the non-junctional resistance of Fig. 2 plotted as $1/R_m(t)$. Only data from the ninth and succeeding minutes is included to remove the effects of the sealing process. The data points were used to compute a best-fitting linear process and the resultant straight line is also plotted.

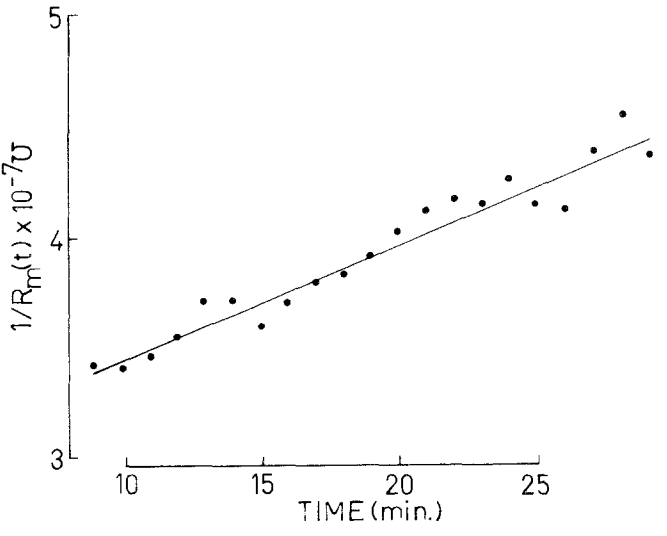

Fig. 3. The values of $R_m(t)$ from the experiment of Fig. 2 plotted as $1/R_m(t)$ versus time. The solid line is a computed best-fitting linear plot with a slope of 5.3×10^{-9} mhos/min and a correlation coefficient of 0.972

The above experiment was performed twenty times and four experiments were acceptable to our criteria for normal development of the embryos. They produced results similar to that illustrated in Figs. 2 and 3. The slopes of the best-fitting linear plots were in the range 5.3 to 13.1×10^{-9} mhos per min. The specific resistance of the cell membranes at this stage has been determined to be approximately $45\,k\Omega\,cm^2$ (DiCaprio *et al.*, 1974; deLaat & Bluemink, 1974). However, if the 70% increase in surface area discussed above is taken into account, a figure of approximately $75\,k\Omega\,cm^2$ is obtained (deLaat & Bluemink, 1974). Using this value together with the linear slopes of conductance *vs.* time which we obtained gives a range of 3.98 to $9.83 \times 10^{-4}\,cm^2/min$ for the rate of increase in membrane area. The linear correlation coefficients for the four plots were in the range 0.971 to 0.985, indicating that the simple model of furrow formation is compatible with the results for $R_m(t)$. Bluemink and deLaat (1973), using light microscopy, have determined that the area of new membrane formed during the first cleavage in *Xenopus* is $1.39 \pm 0.29\,mm^2$. The time during which new membrane is added to the first cleavage furrow is approximately 30 min (Singal & Sanders, 1974) so that the average rate of new membrane formation during this period is $4.6 \times 10^{-4}\,cm^2/min$. Bluemink and deLaat (1974) obtained a figure of $4.0 \times 10^{-4}\,cm^2/min$ for the rate of addition of new membrane to the furrow, but their calculation was based on a model in which the new membrane had very low specific resistance ($1.59\,k\Omega\,cm^2$), and only a small portion of the new membrane was considered to be in electrical contact with the external solution.

Any model for the behavior of the junctional resistance must account for its rapid rise from approximately zero ohms to more than 50% of its final value within the seventeenth minute. Because of the experimental design the component $R_j(t)$ initially represents the resistance of the cytoplasmic bridge through the diminishing disc left by the encroaching new membrane. After the cytoplasmic bridge has completely closed off, the value of $R_j(t)$ represents the resistance of the junction, the structure of which is not completely elucidated. Initially the resistance of the cytoplasmic bridge would be expected to be very small, and it should not assume a detectable value until its diameter is comparable to its length. If new membrane is added to the embryo at a constant rate it is easy to show that final closure of the cytoplasmic bridge from a low resistance condition would be expected to occur in less than 1 min. We therefore interpret the sudden rise in $R_j(t)$ during the seventeenth minute as closure of the cytoplasmic bridge, whose low resistance up to that time would

dominate the observed value even if the junctional resistance between the cells was already established in a different portion of the cleavage furrow. Using the rates of membrane addition computed above the range of times between the onset of the first cleavage and closure of the cytoplasmic bridge would be 26–64 min. The experiment of Fig. 2 commenced at the far advanced groove stage, which is 15 to 18 min after the onset of cleavage (Singal & Sanders, 1974), so that the sudden rise in $R_j(t)$ occurred at about 32 to 35 min after the onset of cleavage, compatible with the calculated rate of membrane addition. The results for $R_j(t)$ are therefore comprehensible in terms of the simple model of cleavage.

Electrical Recording from the Blastocoel

The primitive blastocoel appears during the first cleavage as a dilatation in the cleavage furrow (Kalt, 1971*a*, *b*; Singal & Sanders, 1974). To determine whether current flowing between the embryonic cells passes through the primitive blastocoel, we sought to record directly from the cavity using a microelectrode. Fig. 4 illustrates the result of such an experiment on an eight-cell embryo. Attempts to perform the experiment on embryos at an earlier stage were not successful because of the small size of the cavity compared to the embryo. A current injecting electrode was placed in one of the four animals pole cells of the embryo and the normal 0.5 Hz injecting current was applied to it. A recording electrode was placed in the diagonally opposite animal pole cell and recorded the coupled sinusoidal modulation. A second recording electrode was then attached to the hydraulic micromanipulator and inserted through the first cell at an angle of 45° to the horizontal so that movement of the micromanipulator would cause the electrode to advance into the primitive blastocoel. The figure shows a trace of the measured voltages *vs.* time as the electrode was advanced through the embryo. The moving electrode initially measured a membrane potential of about -15 mV with a superimposed sinusoidal modulation due to the injected current. The fixed recording electrode measured a similar situation in the coupled cell. There followed an abrupt change of the voltage from the moving electrode to zero volts with no detectable sinusoidal modulation. The electrode was then withdrawn until the membrane potential and modulation were again observed and finally the moving electrode was withdrawn completely from the embryo to the surrounding solution where zero volts was again observed. During this process the fixed electrode continued to record the membrane poten-

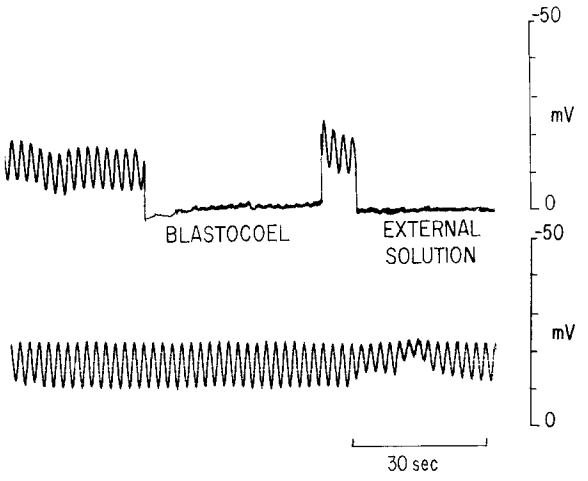

Fig. 4. Electrical recording from the primitive blastocoel of an eight-cell embryo. A current injecting electrode was placed in one of the animal pole cells and a sinusoidal current was applied at 0.5 Hz. A fixed recording electrode was placed in the diagonally opposed animal pole cell and recorded the coupled fluctuations in membrane potential (lower trace). A moving microelectrode was advanced through the first cell where it recorded the sinusoidal modulation of the membrane potential (upper trace) and then into the primitive blastocoel where a potential of zero volts was observed. The moving electrode was then withdrawn through the injected cell and finally out into the external solution where a potential of zero volts was again observed. No significant electrical difference can be seen between the primitive blastocoel and the surrounding saline solution, while electrical coupling between the diagonally opposed cells continued throughout the experiment. The transient decrease in amplitude of the coupled signal at the instant when the moving electrode was removed from the embryo was probably due to partial unsealing of the fixed electrodes during removal of the moving electrode from the embryo

tial and coupled modulation observed initially. A transient decrease in amplitude as the moving electrode was withdrawn was probably caused by partial unsealing of the two fixed electrodes during removal of the moving electrode from the embryo.

We interpret the traces of Fig. 4 to mean that the electrode passed through an animal pole cell and entered the primitive blastocoel, where a potential identical to the external bathing solution was observed with no sinusoidal modulation. No other explanation seems possible since all of the eight cells are strongly electrically coupled to one another at this stage (DiCaprio *et al.*, 1975) and there are no other cavities of significant size. The experiment of Fig. 4 was repeated several times with identical results. In some experiments we continued to advance the moving electrode through the blastocoel until another cell membrane was encountered and a coupled modulation was observed. The distance required to advance the electrode through the region with a potential of zero volts was about

150 μ which agrees well with the size of the blastocoel at this stage observed by scanning electronmicroscopy (DiCaprio *et al.*, 1975).

The absence of membrane potential and sinusoidal modulation in the blastocoel cannot be attributed to a general cessation of coupling since the diagonally opposed cell continued to receive a steady coupled signal throughout the experiment. The blastocoel includes portions of the surfaces of all eight cells in the embryo and yet is not invaded by the currents which are flowing between them. At the same time the blastocoel is clearly connected to the outside solution and so is not electrically sealed off from that solution by very narrow or completely sealed cleavage furrows. It is difficult to see how any mechanism involving large areas of new, low resistance membrane can account for these facts and at the same time directly couple all of the eight cells to one another.

Discussion

The behaviour of the junctional and nonjunctional membrane resistance in *Xenopus* embryos during the first cleavage, and the nonjunctional membrane resistances and capacitances at each of the first three cleavage stages, may be accounted for by a model having the following properties: (1) new membrane is added to the cleavage furrow at a constant rate; (2) the new membrane has identical electrical properties to the original membrane but is much less rough in texture; (3) the furrow and primitive blastocoel are electrically connected to the perivitelline space; (4) electrical coupling between the cells occurs through specialized junctions which are functional at the time of cytoplasmic separation.

The experiments which we have performed on the primitive blastocoel strongly support a model in which the furrow and primitive blastocoel are in direct electrical contact with the perivitelline space. They also preclude electrical coupling of the cells via flow of current through the primitive blastocoel. Further evidence for this view is given by Signal and Sanders (1974) who demonstrated that the entire furrow was accessible to lanthanum ions applied during fixation. The presence of a sealing ring of tight junctions would prevent the penetration of these ions into the deeper regions of the furrow (Revel & Karnovsky, 1967).

deLaat, Luchtel and Bluemink (1973) and deLaat and Bluemink (1974) have also examined the electrical behavior of *Xenopus* embryos during the first cleavage. However, they measured the total input resistance of the embryos and ended their experiments before completion of the first

cleavage. Although they observed a similar reduction in input resistance to that reported here, the time course of the change in membrane resistance which they reported was completely different to that which we have consistently observed. Instead of the step-like decrease in resistance lasting about 5 min which they describe, we have always observed smooth reductions in input resistance, from a value approximating that of an uncleaved embryo, over complete experiments of about 30 min. These changes were seen to occur during the section of the cleavage time when deLaat and Bluemink (1974) reported that all changes in membrane resistance had ceased. It is difficult to account for this difference in results on the same preparation. The step-like reduction in resistance is incompatible with an open furrow model of cleavage since it suggests that the addition of new membrane ceases long before completion of the first cleavage.

The membrane potential, input resistance and input capacitance of one side of a cleaving *Rana pipiens* embryo were measured by Woodward (1968). Unfortunately his experimental results were very variable, as has already been noted by deLaat and Bluemink (1974). Woodward reported large increases in the input resistance which contrasts with the declines observed here and by deLaat and Bluemink (1974) while his measured capacitance for the single cell was about twice that reported here, although this may be a species difference. The increase in membrane capacitance during cleavage was reported by Woodward to be about 3 %, which he suggested was due to electrical isolation of the furrow membranes, although no strong supportive evidence for this was provided.

Slack and Warner (1973) have presented evidence for ionic isolation of the blastocoel at the early blastula stage in *Xenopus*. They also asserted that this isolation commenced at the two-cell stage. However, although intimate intercellular appositions have been demonstrated during early cleavages in *Triturus* (Selman & Perry, 1970) and *Xenopus* (Kalt, 1971*b*; Singal & Sanders, 1974), there is no evidence for the view that they form a sealing ring which electrically isolates the blastocoel from the perivitelline space.

Electrical coupling can occur between embryonic cells of later stages of development which have been physically separated and then placed in contact. This has been demonstrated in *Triturus* (Ito & Loewenstein, 1969; Ito, Sato & Loewenstein, 1974*a*, *b*), in *Xenopus* (Sheridan, 1971) and in *Fundulus* (Bennett, Spira & Pappas, 1972). Since electrical coupling can be demonstrated when the cells are only joined by fine processes it seems unlikely that a cavity lined by low resistance membrane is responsible for

this phenomenon. Ito *et al.* (1974*a*, *b*) presented evidence that specialized membrane appositions are responsible for the coupling.

Evidence for the difference between the properties of old and new membrane has been presented for *R. pipiens* embryos growing in hypertonic solutions (Woodward, 1968) and for *Xenopus* embryos growing in the absence of the vitelline membrane or in the presence of Cytochalasin-B (deLaat & Bluemink, 1974). The changes in membrane resistance with time in these preparations must be treated with caution since the effects of the modifying agents are incompletely understood. Bluemink and deLaat (1973) noted that removal of the vitelline membrane or addition of Cytochalasin-B not only caused eversion of the cleavage furrow but also altered the rate of membrane formation and the total amount of new membrane formed during cleavage. Removal of the vitelline membrane is a traumatic procedure and may well interfere with mechanically fragile structures such as close membrane appositions.

Although we have argued that the primitive blastocoel is not electrically isolated from the perivitelline space during the first two cleavages, we do not believe that this situation prevails throughout early development. On the contrary, it has been reported by Sanders and Zalik (1972) that there is good evidence for sealing tight junctions in the early blastula. In addition we have conducted preliminary experiments which suggest electrical isolation of the blastocoel in early blastulae. Slack and Warner (1973) have presented evidence for a mechanism of blastocoel enlargement which depends upon an active sodium pumping mechanism and a high relative permeability to potassium of the blastocoel walls in early blastulae. It is possible that the transient changes in potassium sensitivity of the membrane potential during earlier stages, which they have reported, are a precursor of this mechanism but that it does not become established until an outer layer of cells sealed from the external medium by tight junctions is formed.

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Erratum

p. 357: Title should read: "The Use of Ionophores for Rapid Loading of Human Red Cells with Radioactive Cations for Cation-Pump Studies", instead of "The Use of Ionophores of Rapid..."