NEW MEMBRANE FORMATION DURING CYTOKINESIS IN NORMAL AND CYTOCHALASIN B-TREATED EGGS OF *XENOPUS LAEVIS*

II. Electrophysiological Observations

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ABSTRACT

The electrical membrane potential (E_m) and electrical membrane resistance (R_m) were measured continuously during the first cleavage of *Xenopus* eggs, using intracellular microelectrodes. A sharp hyperpolarization of E_m and decrease in R_m can be observed from 6 to 8 min after the onset of cleavage. This moment coincides with the onset of the insertion of new membrane (Bluemink and de Laat, 1973) leading to the formation of the interblastomeric membrane during normal cleavage. Removal of the vitelline membrane or exposure to cytochalasin B (CCB) leads to exposure of the entire surface area of the membrane newly formed during cleavage. These conditions allow for a direct measurement of the permeability properties of the new membrane. It was found that under these conditions E_m reaches values about 3 times more negative and R_m reaches values about 1.5–3 times smaller than during normal cleavage. The extent of reduction of R_m can be correlated with the surface area of the newly formed membrane. We conclude that the new membrane has different ionic permeability properties than the pre-existing membrane (most probably a relatively high permeability for K^+ ions). Its mean specific resistance is 1–2 $k\Omega \cdot cm^2$, as against 74 $k\Omega \cdot cm^2$ for the pre-existing membrane. No influence of CCB on the permeability properties of the pre-existing or new membrane could be detected.

INTRODUCTION

It is possible to distinguish two phases in the process of furrow formation in the amphibian cleaving zygote. The cleavage process starts with a local surface constriction due to the action of a contractile microfilament system underlying the cell membrane (Bluemink, 1970, 1971 *a*, 1971 *b*; Kalt, 1971; de Laat et al., 1973; Selman and Perry, 1970). Surface constriction is followed by a process of membrane insertion in the plane of the furrow to complete the separation of the blastomeres (Bluemink, 1970, 1971 *b*; Schechtman, 1937; Selman and Perry, 1970; Selman and Waddington, 1955; Zotin, 1964). However, direct morphological evidence regarding the process of membrane formation during cytokinesis is scarce (see Bluemink and de Laat, 1973), and data on the physiological properties of this membrane material are even more scanty. Woodward (1968) made an analysis of the changes in electrical membrane characteristics in *Rana pipiens* eggs dividing normally and in eggs undergoing furrow regression due to exposure to hypertonic salt solutions.

Mainly on the basis of the increase in membrane capacitance during furrow regression, he suggested that during cleavage new membrane is introduced between the blastomeres, and that this membrane has a low resistance compared to the pre-existing outer membrane.

The finding that in *Xenopus* eggs electrical membrane characteristics start to change at 6–8 min after the onset of first cleavage (de Laat et al., 1973), and the hypothesis that these changes are brought about by the incorporation of new membrane in the bottom of the cleavage furrow have formed the basis of our recent work. The foregoing study (Bluemink and de Laat, 1973) was devoted to the investigation of the ultrastructural changes connected with new membrane formation, to the rating of new membrane formation by surface measurements under different conditions, and to the direct demonstration of *de novo* surface formation by surface-marking experiments. A temporal correlation was found between the morphological signs of new membrane formation and changes in the electrical membrane properties. This provides evidence for the above hypothesis. Measurements of the geometrical surface area of the newly formed surface showed that the rate of new membrane formation is influenced by environmental conditions. When the vitelline membrane is removed the entire new surface is exposed to the medium, then visible as unpigmented surface material (Fig. 7). This area of newly formed surface is smaller than the interblastomeric surface formed in normally cleaving eggs. The exposure of unpigmented surface area to the medium occurs also after cytochalasin B (CCB)-induced furrow regression. Both treatments enable one to investigate directly the properties of the unpigmented surface, which in normally cleaving eggs will form the intercellular membrane, and as such, is relatively inaccessible to the investigation of permeability properties.

In the present study an analysis was made of the relation between the changes in membrane properties and the insertion of new membrane material during the first cytokinetic cycle. Using microelectrode techniques, the electrical membrane potential (E_m) and electrical membrane resistance (R_m) were measured in eggs cleaving inside and in the absence of the vitelline membrane, and in eggs undergoing CCB-induced furrow regression. The possible influence of CCB on the ion-permeability properties of the membrane (Bluemink, 1971 *b*; Hammer et al., 1971; de Laat et al., 1973) was studied and calculations were made of the specific membrane resistance of the pre-existing and newly formed membrane.

MATERIALS AND METHODS

Eggs of *Xenopus laevis* were derived from hormonally stimulated couples, chemically decapsulated, and handled as described before (de Laat et al., 1973). Eggs were kept in Steinberg solution (Steinberg, 1957) unless otherwise indicated. Experiments were carried out at room temperature (20–24°C). For some experiments the vitelline membrane was removed with forceps.

Electrical Measurements

E_m and R_m were measured continuously during egg cleavage, using microelectrode techniques. Current-passing and voltage-recording glass microelectrodes were filled with 3 M KCl and had 5–10 MΩ DC resistance. Tip potentials were smaller than 1 mV. Microelectrodes were connected to Pt-Ag-AgCl electrodes via Steinberg solution bridges. When the total drift of electrode potentials was greater than 1 mV during one experiment (usually 30–60 min), the results were not used. Generally, two voltage-recording microelectrodes were used as a control for isopotentiality of the cytoplasm. Intracellular voltages were measured with respect to an indifferent Pt-Ag-AgCl electrode in the bath by means of differential preamplifiers (Transidyne MPA-6, Transidyne General Corp., Ann Arbor, Mich.). The bath was grounded via a separate Pt-Ag-AgCl electrode. Constant current pulses of 1.2-s duration and 3×10^{-8}-A amplitude were delivered every 10 s by a constant current stimulator. The polarity of the pulses was alternated to prevent net current flow through the electrodes and the cell membrane. The current was measured by means of a current-to-voltage converter in the ground return of the circuit (Fig. 1).

In most cases the microelectrodes were introduced into the egg about 10 min before the onset of first cleavage. This stage can be determined from pigment movements in the animal egg cortex resulting in a more "granular" pigmentation pattern (Rzehak, 1972). The voltage-recording microelectrodes were inserted first. When the measured E_m reached a stable value (after ca. 3 min), the current-passing microelectrode was inserted. Both the intracellular voltages and the applied current were monitored on an oscilloscope (Tektronix 565, Tektronix, Inc., Beaverton, Ore.) and recorded on a pen-recorder (Hellige 19, Fritz Hellige & Co., GMBH; Freiburg in Breisgau, B.R.D.). Every second, in phase with the front edges of the current pulses, the outputs of the preamplifiers and of the current-to-voltage

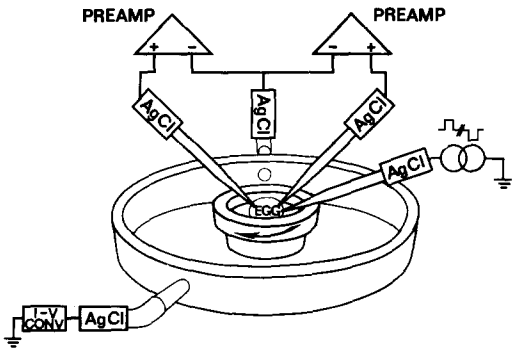


FIGURE 1 Schematic diagram of the Perspex measuring chamber and the electrode arrangement used in the present experiments. Not to scale.

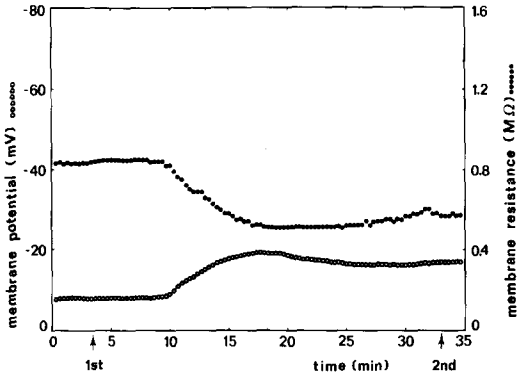


FIGURE 2 Changes in E_m and R_m during the first cleavage of a *Xenopus* egg within the vitelline membrane. The arrows indicate the onset of the first and second cleavage, respectively.

converter were simultaneously digitally measured and recorded on punch tape (Facit 4070, Facit A. B., Atvidaberg, Sweden). Calculations and plots of E_m and R_m were made on a Philips X-8 computer (Philips Nederland N. V., Eindhoven, The Netherlands) at the Academic Computer Centre, Utrecht. For further details of the method used, see de Laat et al. (1973).

Replacement of Bathing Medium

For some experiments it was necessary to replace the bathing medium during a measurement by a medium of different composition. The egg was placed in a small hemispherical hole (diameter 1.5 mm, depth 0.5 mm) on top of a cylindrical elevation in the middle of the Perspex measuring chamber (Fig. 1). The measuring chamber contained about 20 ml of medium. A ring could be turned up and down the elevation. When it was turned up, about 95% of the bathing medium could be removed while the egg remained in the original medium within the ring. After the introduction of a new solution the ring was turned down, resulting in a rapid exchange of the medium surrounding the egg. This method was preferred over the reciprocal infusion-withdrawal pump system (see e.g. Tupper, 1972), since it requires less time to obtain the same dilution and gives a rapid exchange of solutions in the egg environment.

RESULTS

Normal Cleavage

During first cleavage of *Xenopus* eggs within the vitelline membrane, E_m and R_m invariably change according to a characteristic pattern (Fig. 2). (See also de Laat et al., 1973.) After initial wound closure around the inserted microelectrodes, which is reflected in an increase in R_m and a relatively small increase in E_m (not shown: the process of wound closure precedes the time span shown in the figure), E_m remains nearly constant during the first 6–8 min of cleavage. In the next 10 min it becomes about 10 mV more negative, while during the second half of cleavage it often shows a small decrease (1–3 mV) preceding the onset of the second cleavage. R_m shows a pattern almost inverse to that of E_m. Being constant initially, it drops drastically from 7 till 17 min after the onset of first cleavage. Often a small increase precedes the onset of second cleavage. The absolute values of both E_m and R_m vary from egg to egg, the variation in resistance being relatively great (Table I). However, the pattern of changes is well defined. The change in E_m and R_m invariably starts between 6–8 min after the onset of first cleavage. The small drop in E_m coincides with the moment at which the blastomeres become apposed more closely. The potentials recorded by two microelectrodes inserted in different regions of the animal part of the egg never showed significant differences, indicating isopotentiality of the cytoplasm and complete electrical coupling during the first cleavage.

From these experiments it can be concluded that the electrical membrane properties change from a well-defined moment onwards during the cleavage cycle. This may be due to a change in permeability properties of the pre-existing cell membrane, to the insertion of new membrane material with permeability properties different from those of the pre-existing membrane, to a rapid change in intracellular ion activities, or to any combination of these factors.

TABLE I

The Membrane Potential and Membrane Resistance at the Onset of the First and Second Cleavages under Various Conditions

Condition	Onset of first cleavage		Onset of second cleavage	
	Membrane potential	Membrane resistance	Membrane potential	Membrane resistance
	mV	*kΩ*	*mV*	*kΩ*
+ Vitelline membrane − CCB	−9.7 ± 0.6 (25)	852 ± 48 (22)	−20.3 ± 0.8 (20)	488 ± 28 (17)
− Vitelline membrane − CCB	−9.8 ± 0.7 (8)	901 ± 62 (8)	−60.0 ± 2.5 (7)	336 ± 31 (7)
+ Vitelline membrane + CCB	−10.8 ± 0.5 (7)	779 ± 43 (6)	−60.9 ± 3.4 (6)	165 ± 10 (6)

The results are presented as mean ± standard error (number of observations in brackets).

Intracellular Na^+, K^+, and Cl^- activities have been measured during cleavage, using ion-selective microelectrodes.[1] Although minor but significant changes can be observed, they cannot account for the change in E_m. Morphologically it can be seen that new membrane material is inserted into the pre-existing membrane (Bluemink and de Laat, 1973) from approximately 7 min after the onset of first cleavage onwards. During normal cleavage, only a minor part of this material is exposed to the outer medium, as can be judged from the surface area of the visible, unpigmented surface in the furrow (Fig. 6). The major part forms the intercellular membrane of the two blastomeres.

It might be possible to distinguish between the various possibilities mentioned above if one could force the new membrane material to expose its entire surface to the surrounding medium. If the altered membrane properties are due to the newly formed membrane only, exposure of more of its surface should lead to a greater alteration of the measured E_m and R_m. In cleaving eggs devoid of their vitelline membrane the two blastomeres tend to separate under gravity and expose completely their newly formed membrane to the outer medium (Fig. 7). (See also Bluemink and de Laat, 1973; de Laat et al., 1973.) Eggs exposed to CCB show furrow regression after the initial contraction. Beginning approximately 7 min after the onset of cleavage new membrane material is inserted into the outer surface along the original plane of cleavage. (See also Bluemink, 1971 *a*, 1971 *b*; Bluemink and de Laat, 1973; Hammer et al., 1971; Selman and Perry, 1970.) This results in a meridional band of unpigmented surface surrounding the egg (Fig. 8).

[1] de Laat, S. W., and R. F. D. Buwalda. Manuscript in preparation.

Cleavage in the Absence of the Vitelline Membrane

As pointed out above, the membrane forming the interblastomeric surface in normally cleaving eggs is exposed to the outer medium in eggs cleaving in the absence of the vitelline membrane. The pattern of changes in E_m and R_m is qualitatively similar to that of normally cleaving eggs, but the changes themselves show great quantitative differences (Fig. 3). By comparing the mean E_m and mean R_m in eggs cleaving normally and in eggs cleaving in the absence of the vitelline membrane using the Student's t test, it can be shown that removal of the vitelline membrane does not significantly alter these parameters at the onset of first cleavage ($P > 0.50$). Being constant initially, E_m and R_m change drastically from approximately 7 min after the onset of cleavage onwards. Within 10 min E_m reaches a value about three times more negative than in eggs cleaving insider the vitelline membrane, while R_m becomes about 1.5 times smaller. During the second half of cleavage E_m

and R_m remain more or less constant, although in some cases minor fluctuations can be observed (Fig. 3).

At the onset of second cleavage both E_m and R_m differ significantly from the values obtained in normally cleaving eggs ($P < 0.001$ and $P < 0.005$, respectively; see also Table I). Removal of the vitelline membrane does not significantly alter the electrical properties of the pre-existing membrane before cleavage. It seems unlikely that removal as such would alter the pre-existing membrane during the process of cleavage. Thus, it can be concluded that exposure to the medium of a greater area of inserted new membrane material results in an enhanced alteration of the electrical properties of the outer membrane. Apparently the new membrane has ion-permeability properties different from those of the pre-existing membrane.

Cleavage in the Presence of CCB

It has been suggested previously (Bluemink, 1971 *b*, Manasek et al., 1972) that CCB might interfere with ion-permeability properties of the cell membrane. No evidence supporting this possibility has been found so far. CCB injected into *Xenopus* eggs immediately beneath the middle of the furrow 3–4 min after the onset of cleavage leads to local furrow regression and the appearance of an unpigmented surface area, but does not alter E_m or R_m during cleavage (de Laat et al., 1973).

To investigate whether exposure to externally applied CCB influences the electrical membrane properties, and to examine the permeability properties of the new membrane formed during CCB-induced furrow regression, E_m and R_m were measured in eggs dividing within their vitelline membrane in the presence of CCB. In most measurements the voltage-recording and current-passing microelectrodes were inserted into the egg 20–30 min before the onset of cleavage, the bathing medium being Steinberg solution. After stabilization of the observed E_m and R_m, the medium was replaced by Steinberg solution containing 10 μg CCB/ml and 1% dimethylsulfoxide (DMSO). In some experiments the microelectrodes were inserted while the egg was in the CCB-Steinberg solution. CCB affects the process of wound closure around the microelectrode tip. Normally, closed wounds do not reopen upon addition of CCB to the medium. However, when the microelectrodes are inserted into eggs already exposed to CCB, wounds sometimes fail to close, resulting in leakage between the cytoplasm and the medium. The chances for a successful measurement are improved when penetration of the cell membrane is perpendicular to the cell surface, resulting in a tight, small, circular wound. Wound closure is then reflected in a contraction (Bluemink, 1972) around the microelectrode tip and an increase in measured R_m (de Laat et al., 1973). An example of a successful experiment is given in Fig. 4.

The striking result is that E_m and R_m change according to a pattern essentially the same as observed in eggs cleaving without the vitelline membrane in the absence of CCB. Again, E_m and R_m remain constant during approximately the first

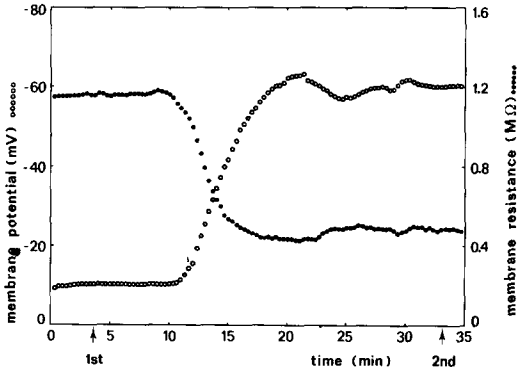


FIGURE 3 Changes in E_m and R_m during the first cleavage of a *Xenopus* egg devoid of the vitelline membrane. The arrows indicate the onset of the first and second cleavage, respectively.

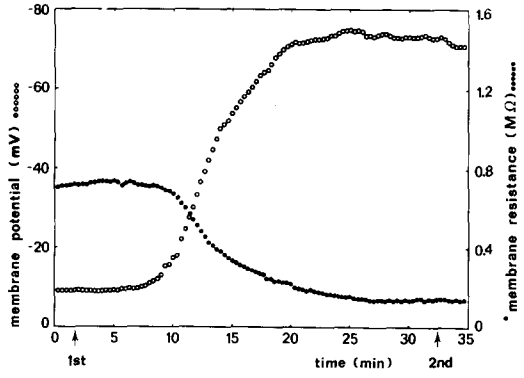


FIGURE 4 Changes in E_m and R_m during the first cleavage of a *Xenopus* egg within the vitelline membrane, exposed to 10 μg CCB/ml. The arrows indicate the onset of the first and second cleavage, respectively.

7 min of cleavage. Comparison of E_m and R_m at the onset of first cleavage in CCB-treated eggs with those in untreated eggs cleaving inside the vitelline membrane (Table I) yields no statistical differences ($P > 0.20$ and $P > 0.40$, respectively). The same holds for a comparison with eggs devoid of their vitelline membrane ($P > 0.20$ and $P > 0.10$, respectively). Apparently CCB does not influence the ionic permeability of the pre-existing membrane. This is in accordance with our previous findings (de Laat et al., 1973).

From approximately 7 min after the onset of cleavage onwards, the observed changes are similar to those in eggs cleaving in the absence of the vitelline membrane with one exception. R_m decreases over a longer period and reaches a lower level at the onset of second cleavage than it does in eggs devoid of their vitelline membrane in the absence of CCB ($P < 0.001$), but no statistical difference is seen in E_m at the onset of second cleavage ($P > 0.20$) (see also Table I). As a control, E_m and R_m were measured in a series of eggs exposed to 1% DMSO in Steinberg solution. Such eggs show a completely normal cleavage and the measured parameters change as in normally cleaving eggs.

In a final series of experiments E_m and R_m were measured in eggs cleaving outside the vitelline membrane while exposed to Steinberg solution containing 10 μg CCB/ml and 1% DMSO. Such an egg shows a furrow formation during the first 6–8 min of cleavage followed by complete furrow regression. The egg flattens strongly against the substrate, and a wide band of unpigmented new surface appears in the original cleavage plane

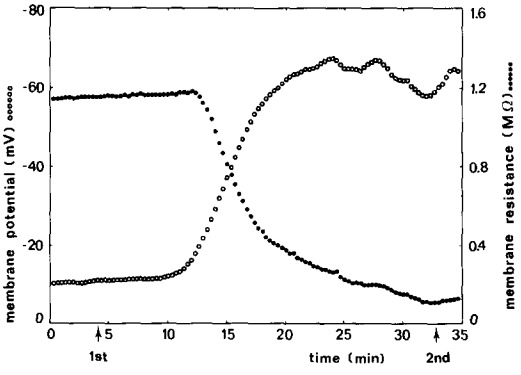


FIGURE 5 Changes in E_m and R_m during the first cleavage of a *Xenopus* egg devoid of the vitelline membrane, exposed to 10 μg CCB/ml. The arrows indicate the onset of the first and second cleavage, respectively.

(see Figs. 4–6 of Bluemink and de Laat, 1973). Measurements made upon such eggs are technically relatively difficult. Small surface injuries are easily provoked during handling of eggs outside the vitelline membrane, and these often do not heal completely in the presence of CCB. Only three successful experiments were carried out. Although this is not enough for a reliable statistical comparison, a qualitative analysis can be made. Fig. 5 shows the results of one such experiment. The changes in E_m and R_m are similar to those in eggs inside the vitelline membrane exposed to CCB. However, the drop in R_m seems to be relatively greater.

Specific Membrane Resistance of the Uncleaved Egg

By combining surface area measurements of the uncleaved egg with measurements of R_m in such eggs, one can obtain an estimate of the specific membrane resistance. It had been found that the mean geometrical surface area of uncleaved *Xenopus* eggs inside the vitelline membrane is 5.11 mm² (Bluemink and de Laat, 1973). The mean R_m is 852 kΩ (Table I). Without making corrections for surface foldings, the specific membrane resistance would be 43.5 kΩ·cm². However, scanning electron microscopy and thin-section transmission show the cell membrane to be very rough (Figs. 9 and 10). Measurements of the distance between two points on the surface following the contour of the membrane yield a length of about 30% in excess of the linear distance (Bluemink and de Laat, 1973). This means that the real surface area is about 1.7 times the measured geometrical surface area and equals 8.69 mm². Using this number, the specific membrane resistance of the uncleaved egg can be put at 74 kΩ·cm².

Specific Membrane Resistance of the New Membrane

It is assumed that the change in E_m and R_m during cleavage arises from the insertion of new membrane material having different ionic permeability properties than the pre-existing membrane. The quantitative differences in these changes due to exposure to CCB or to removal of the vitelline membrane could originate from the differences in surface area of inserted new membrane exposed to

the medium. If this is so, then the specific membrane resistance of the new membrane formed during cleavage in the absence of the vitelline membrane or in the presence of CCB should be equal. Neither treatment alters R_m of the uncleaved egg. By assuming that R_m of the pre-existing membrane remains constant throughout cleavage, it is possible to calculate the resistance of the new membrane inserted into the outer surface, because the new membrane will form a resistance parallel to the resistance of the membrane of the uncleaved egg.

For those eggs in which R_m was measured continuously during cleavage, the resistance of the new membrane at the onset of the second cleavage was calculated. In eggs cleaving in the absence of the vitelline membrane the mean ± SE of the resistance of the new membrane is 553 ± 66 kΩ (n = 7), while in eggs cleaving inside the vitelline membrane in the presence of CCB it is 210 ± 15 kΩ (n = 6). The geometrical surface area of the new membrane had been found to be 0.33 ± 0.02 mm² (n = 11) and 0.65 ± 0·04 mm² (n = 15), respectively (Bluemink and de Laat, 1973).

Multiplication of the mean resistance with the mean surface area gives an estimate of the specific resistance of the new membrane. In eggs cleaving outside the vitelline membrane this value is 1.82 kΩ·cm², while in eggs exposed to CCB it is 1.36 kΩ·cm². No corrections for surface foldings have to be made, since the new membrane has an almost smooth appearance (Figs. 9 and 11). A direct statistical comparison of these values is not possible, since different eggs were used for resistance and surface area measurements. However, knowing the variances of both the resistances and the surface areas, it seems unlikely that the difference between the two values is significant. As an estimate their average can be used: 1.59 kΩ·cm².

Using this calculated specific resistance, it is possible to estimate the surface area of new membrane exposed to the outer medium after normal first cleavage. The resistance of the new surface area in the cleavage furrow that is exposed to the outer medium can be calculated as indicated above. Its mean ± SE is 1148 ± 104 kΩ(n = 17). Using the previously estimated specific resistance of the new membrane of 1.59 kΩ·cm², the surface area of new membrane formed in the normal cleavage furrow would be 0.14 mm². This is 2.7% of the total geometrical surface area of the uncleaved egg and 10% of the total geometrical surface area of new membrane formed during first cleavage (Bluemink and de Laat, 1973). The remaining 90% will form the interblastomeric surface.

DISCUSSION

The course of E_m and R_m during the first cleavage of *Xenopus* eggs has been measured in eggs under three different conditions: inside the vitelline membrane, in the absence and in the presence of CCB; and without vitelline membrane, in the absence of CCB. The last two conditions permitted a direct study of the properties of the newly formed membrane, since this membrane is inserted as part of the outer surface. The previous study (Bluemink and de Laat, 1973) had shown that this new surface is formed *de novo* and not at the expense of the pre-existing surface. Furthermore, ultrastructural observations had indicated that the insertion of the membranous material starts between 4 and 8 min after the onset of the first cleavage.

Our present results show that the electrical membrane properties start to change at approximately 7 min after the onset of cleavage under all conditions used. This moment coincides with the structural changes which accompany the appearance of new membrane. Removal of the vitelline membrane or exposure to CCB does not lead to an alteration in the electrical properties of the pre-existing membrane. For the former treatment this would, anyhow, not have been expected. Nevertheless, the hyperpolarization of E_m and decrease in R_m during cleavage are significantly greater under these conditions than in normally cleaving eggs. At least in the case of vitelline membrane removal, the most obvious explanation for this difference is the exposure of a greater area of newly formed membrane having ionic permeability properties different from those of the pre-existing membrane. On the basis of this assumption and the measurements of the geometrical surface area of the new membrane (Bluemink and de Laat, 1973), the mean specific membrane resistance of the new membrane is calculated to be 1.82 kΩ·cm². This is comparable to the value of 950 Ω·cm² computed for the specific membrane resistance of the new membrane in *Rana pipiens* eggs (Woodward, 1968) and to the specific resistance of the cell membrane of many tissue cells (Plonsey and Fleming, 1969). The general appearance of the

new membrane is that of a smooth surface (Fig. 11). The lumina of the open cisternae of the endoplasmic reticulum (ER) reported previously (Bluemink and de Laat, 1973) can be considered as extracellular, and consequently, the membrane of this part of the ER system is an integral part of the newly formed membrane. In calculating the specific membrane resistance of the new membrane, no correction has been made for a possible increase in surface area due to these open cisternae. No estimates are available for the number of cisternae open at any given moment, but it seems to be rather small. If the open necks of the cisternae would be large enough to constitute a low-resistance pathway, and if the membrane of the ER would have a low specific resistance, then the specific resistance of the new membrane would be higher than calculated. The membrane of the uncleaved egg is relatively impermeable to ions as judged by its specific resistance of 74 $k\Omega \cdot cm^2$ (corrected for surface foldings).

With respect to their membrane properties, CCB-treated eggs behave roughly like eggs cleaving in the absence of the vitelline membrane. The main difference is the prolonged decrease in R_m and its lower level at the onset of second cleavage. These effects become more pronounced when CCB is applied to eggs cleaving outside the vitelline membrane. There is good agreement between the calculated specific membrane resistances of the new membrane after CCB treatment and after removal of the vitelline membrane. Assuming the specific resistance of the new membrane to be constant throughout the process of insertion, it is possible to use the change in R_m for calculating the growth of new membrane area as a function of time. If this is done for eggs cleaving without the vitelline membrane in the absence of CCB (Fig. 3) and for eggs cleaving inside the vitelline membrane in the presence of CCB (Fig. 4), it is found that the growth rate is constant in both cases and has a value of about 0.04 mm^2 per min. However, the duration of growth is 7–10 min for the former treatment and 15–17 min for the latter. We have no explanation for this difference. On the basis of the same assumption, eggs cleaving without the vitelline membrane in the presence of CCB show a different membrane growth pattern. During the first 15–17 min the growth rate is constant and of the same magnitude as under the conditions mentioned earlier. This period is followed by a period of exponential increase in growth rate during the latter part of cleavage, resulting in a much larger new membrane area (Bluemink and de Laat, 1973). The cause of this prolonged and increased growth is unknown.

Our experiments do not provide evidence that CCB does alter the ionic permeability properties of the membrane, a possibility advanced by Bluemink (1971 *b*) and by Manasek et al. (1972). CCB interferes with the contractile system responsible for furrow formation (de Laat et al., 1973) and deranges the rate and the direction of growth of new membrane (Bluemink and de

FIGURE 6 End of first cleavage, egg inside the vitelline membrane. The two blastomeres are closely apposed; hardly any unpigmented surface (arrow) is visible. × 35.

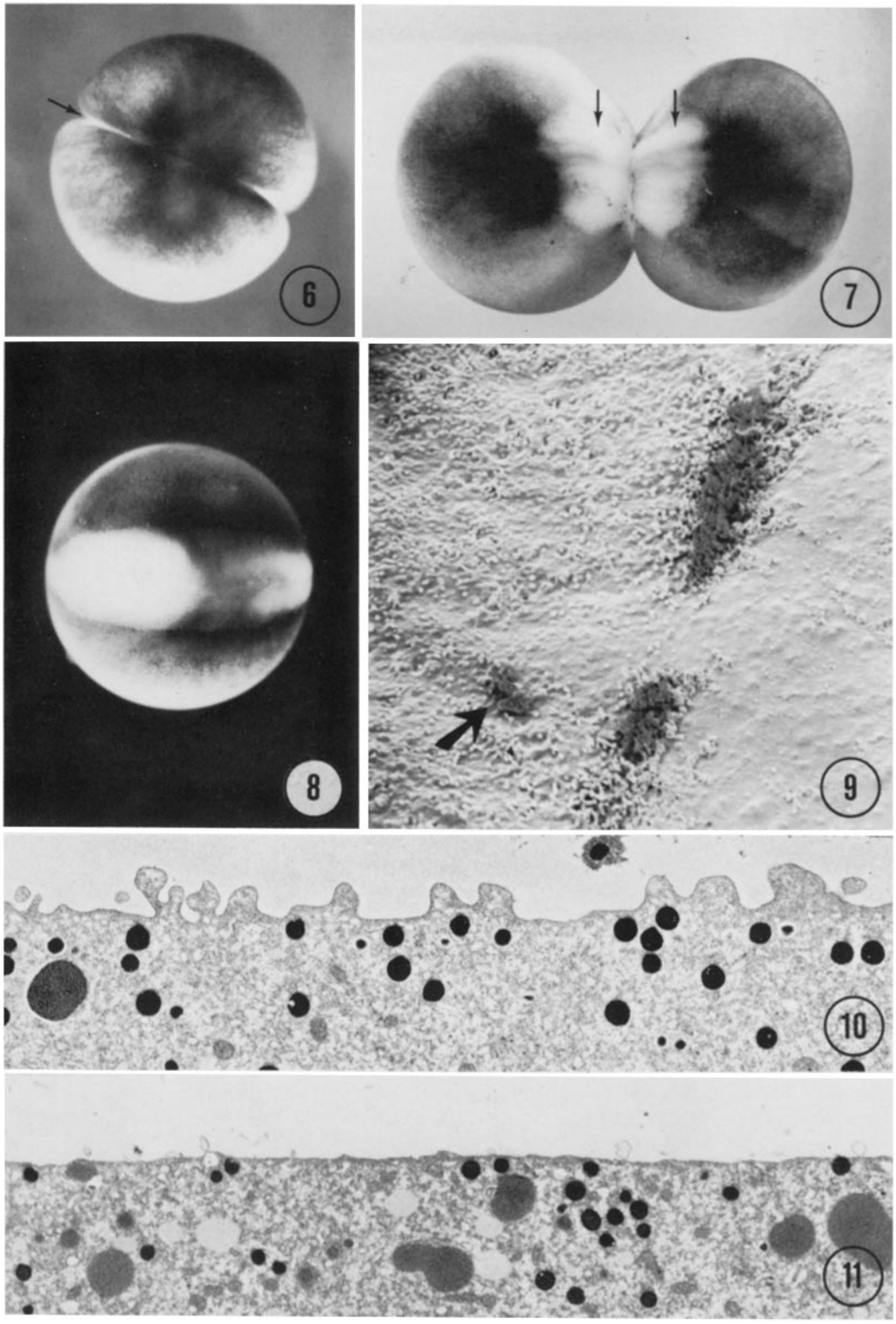
FIGURE 7 End of first cleavage, egg without vitelline membrane. The two blastomeres have moved apart; unpigmented surface (arrows) is exposed to the medium. × 35.

FIGURE 8 End of first cleavage, egg inside the vitelline membrane showing complete CCB-induced furrow regression. Unpigmented surface has been inserted in the former furrow area. The edge of this area is shown in the next figure. × 35.

FIGURE 9 Scanning electron micrograph. Borderline between pigmented rough (left) and unpigmented smooth surface (right) is marked by a ridge. The smooth surface extends across the ridge into the rough surface area. Dislocated fragment of the ridge (arrow). × 900.

FIGURE 10 Pigmented rough surface of the animal hemisphere outside the furrow region at the onset of first cleavage. × 6600.

FIGURE 11 Egg exposed to 10 μg CCB/ml at the onset of first cleavage, fixed 11 min later. Unpigmented smooth surface along the regressed furrow, i.e., new membrane area. × 6600.
The *Xenopus* eggs were prepared for electron microscopy following the method described previously (Bluemink and de Laat, 1973).



Laat, 1973). Whether both effects are due to a common cause, or whether one effect causes the other is unknown.

Knowing that the altered permeability properties are due to the new membrane, the sequence of changes in E_m and R_m during normal cleavage (Fig. 2) becomes understandable. During approximately 7 min of cleavage the furrow is established, most probably by the action of a microfilamentous contractile system underlying the cell membrane (Bluemink, 1970, 1971 *a*, 1971 *b*; Kalt, 1971; Selman and Perry, 1970). The increase in total geometrical surface area necessary for furrow formation results from smoothing out of the pre-existing rough surface, as can be judged by the unaltered E_m and R_m during this phase. From that moment on, the interblastomeric surface begins to be formed, a process not yet completed at the onset of the second cleavage (Bluemink and de Laat, 1973). A small fraction of this newly formed membrane is inserted into the walls of the cleavage furrow itself during the first 10 min of new membrane formation. It is visible as unpigmented surface at the bottom of the furrow. This fraction is responsible for the rapid hyperpolarization of E_m and decrease in R_m. Also, in scanning electron micrographs, patches of smooth surface can be seen to be inserted into the pre-existing, rough surface (Fig. 9). During the latter part of the cleavage process the distance between the walls of the cleavage furrow is reduced, and the two blastomeres tend to flatten against and adhere to each other. The degree of adhesion is rather variable among different eggs. This process results in the observed small depolarization of E_m and small increase in R_m towards the onset of the second cleavage, which is due to a reduction of the exposed area of new membrane. It is important to realize that changes in E_m and R_m similar to those described for the first cleavage can be observed during the succeeding cleavage cycles.

To determine which ion permeabilities are predominant in the new membrane, E_m, R_m, and intracellular Na^+, K^+, and Cl^- activities were measured simultaneously in dividing eggs bathed in media of different ionic composition.[1] The ion activities were measured with ion-selective microelectrodes. From this study it was concluded that the new membrane has a relatively high permeability for potassium ions. During cleavage the membrane potential shifts towards the equilibrium potential for potassium (in Steinberg solution: $E_{K^+} = -115$ mV).

Our results may have some important consequences for the concept of intercellular communication in early amphibian development. It is a well-established fact that the blastomeres in early cleavage stages are electrically coupled (Ito and Hori, 1966; Palmer and Slack 1970; Woodward, 1968). In our measurements the coupling ratio V_2/V_1 (Loewenstein, 1966) was always greater than 0.95 during first cleavage. Nothing else can be expected since the intercellular membrane is not yet completed at the onset of second cleavage, and a cytoplasmic bridge still connects the two blastomeres (Bluemink and de Laat, 1973) However, after the completion of the intercellular membrane, its relatively low specific resistance, due to high potassium permeability, will result in an appreciable coupling ratio without it being necessary to assume that specialized membrane junctions are involved in the transport of ions across the intercellular membrane. According to Sanders and Zalik (1972) such junctions are indeed absent at these stages.

The hyperpolarization of the membrane accompanied by an increased conductance, as we observed, seems to be a general phenomenon during cleavage stages of amphibian embryos. Palmer and Slack (1970) reported similar changes during early development of *Xenopus* embryos. However, no detailed analysis of the course of changes during a single cleavage cycle was presented by them, and as a consequence, no direct relation between the observed changes and new membrane formation could be detected. Their suggestion that the hyperpolarization could be due to a fall in intracellular sodium concentration in combination with a change in the relative permeability of the surface membrane to sodium and potassium now appears to be unrealistic, since no such changes in sodium activity have been observed during first cleavage.[1] Ito and Hori (1966) performed a similar but less detailed study on *Triturus* eggs, but no attempt was made to explain the observed increase in membrane potential.

The situation during first cleavage of *Rana pipiens* eggs has been studied in more detail by Woodward (1968). In eggs dividing normally, the membrane capacitance remains relatively constant while E_m and R_m change according to a pattern roughly similar to that described here for *Xenopus* eggs. However, the pattern is obscured by many rather large fluctuations, which could be artifactual (incomplete wound healing). In eggs undergoing furrow regression due to exposure to

hypertonic salt solutions, he observed an increase in membrane capacitance and a greater alteration in E_m and R_m. Mainly on the basis of the change in membrane capacitance, Woodward suggested that new membrane is introduced between the blastomeres, and that this membrane has a low resistance compared to the pre-existing membrane. E_m was found to be sensitive to the potassium concentration in the bathing medium. He concluded that the new membrane has selective permeability to potassium ions, but no intracellular ion activities were determined.

In nonamphibian embryos, early cleavage is also associated with a hyperpolarization of the membrane (e.g. *Fundulus*, Bennett and Trinkaus, 1970; *Asterias*, Tupper, 1972; *Fucus*, Bentrup, 1970; Weisenseel and Jaffe, 1972). In the latter two species, it was shown that the change in E_m is related to an increase in potassium permeability. However, no direct relation with the process of egg cleavage was reported.

Whether these changes in membrane properties have any significance for embryonic development still has to be shown, and at this time one can only speculate. The finding that cyclic cell surface alterations (Burger et al., 1972) as well as changes in E_m as such (McDonald et al., 1972) can influence the rate of DNA synthesis suggests that postmitotic new membrane formation may be instrumental in development.

We thank Dr. S. B. Carter, Imperial Chemical Industries, England for a gift of cytochalasin B, Miss E. S. Marcus and Mr. E. van Voorst for technical assistance, and Miss E. Bartova, Mrs. I. Aleven, and Mr. L. Boom for preparing the drawings and prints. We are grateful to Dr. J. Faber and Prof. P. D. Nieuwkoop for reading the manuscript.

Received for publication 7 May 1973, and in revised form 9 October 1973.

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