Contractile responses at the surface of an amphibian egg

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During the course of experiments involving the application of polyelectrolytes to amoebae (Gingell, 1967 a) it became apparent that highly charged cations are capable of affecting cellular motility. In some circumstances localized applications apparently result in contraction of the cell surface. Korohoda, Forrester, Moreman & Ambrose (1968) subsequently described a reversible charge-dependent response at the surface of isolated amoeba nuclei, which they attributed to nuclear membrane contraction and expansion. Dr Korohoda suggested to me that the surface of *Xenopus laevis* eggs might be expected to provide a conveniently large experimental system since any contraction would be readily visible.

The localized application of cationic polyamino acid solutions close to the cell surface had a remarkable effect: the treated region coalesced into a dense black spot, blackening being due to pigment granule accumulation (Gingell, 1967 a). Electron microscopy showed that the effect was not in this case due to contraction of the surface membrane, but to the activity of a thin layer of cortical cytoplasm just beneath the plasma membrane, where contraction dragged pigment granules centripetally and caused extensive folding of the overlying surface membrane. What is perhaps even more remarkable is that the contractile wound closure response of the egg cell surface, described by Holtfreter and attributed by him to the action of a surface coat in a series of publications (Holtfreter, 1943 a, b; 1946, 1947) appears identical in the electron microscope, showing that wound healing contraction takes place in the cortical cytoplasm and not in the membrane or surface coat.

Experiments which will be described in this paper as well as work already published (Gingell & Palmer, 1968) show that increasing the membrane permeability of *Xenopus* eggs by either adsorbing polycations at the cell surface or simply making a wound in the surface with a fine tungsten probe, allows Ca$^{2+}$ ions to enter and interact with the cortical contractile system, giving rise to a localized and reversible contraction. The cortical mechanism shares a number of features in common with muscle.

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MATERIAL AND METHODS

Jelly layers and the vitelline membrane were removed from fertilized and unfertilized eggs of *Xenopus laevis* with fine forceps in full strength Steinberg's medium, under a Stereozoom microscope at approximately neutral pH. Steinberg's solution contains the following: 58.6 mM-NaCl; 0.67 mM-KCl; 0.34 mM-Ca(NO₃)₂ · 4 H₂O; 0.83 mM-MgSO₄ · 7 H₂O; 1.10 mM-HCl; 4.63 mM 'Tris' buffer (trishydroxymethylaminomethane) in glass-distilled water. Transference of eggs without vitelline membranes was done with a teat pipette of large bore with a flamed orifice, taking great care not to allow friction between egg surface and the glass walls and avoiding contact with air-water interfaces, which result in egg lysis. It was often more convenient to change solutions in a solid watchglass containing an egg rather than remove the egg to another watchglass. Initially, only undamaged eggs were used, but it was found that those which had healed minor wounds or even major ones, responded similarly. For electron microscopy, only undamaged eggs were used and these were not pipetted prior to fixation. For the short term experiments, satisfactory development was attained on glass, plastic or agar-covered surfaces, though later cleavages invariably gave rise to an abnormal blastula. Test solutions were applied locally by means of a fine glass pipette.

Histone sulphate, poly-L-lysine HBr of molecular weights (M) 2600 and 150000, poly-L-ornithine of M 90000 and sodium polyglutamate, M 68000 were obtained from Sigma Co., while poly-L-lysine HBr M 50000 was obtained from Koch-Light Laboratories. Purified ionic detergents were a gift of Professor E. A. Barnard. Polyelectrolyte, protein and detergent solutions were freshly prepared in glass distilled water and the pH was adjusted as necessary with N/20-NaOH or N/20-HCl to the required value.

Electron microscopy

Specimens for electron microscopy were fixed in three different ways.

(1) The method of Byers & Porter (1964) was used by these authors for detecting filaments in chick embryo cells. Fixation, rinsing, postosmification and initial dehydration were performed in Sorensen's phosphate-biphosphate buffer containing 1.5 mM-CaCl₂. Fixation by 3% glutaraldehyde in 0.05 M buffer for 1 h was followed by two changes of 0.1 M buffer, of 15 min each. After 45 min postosmification in 1% osmium tetroxide made up in 0.1 M buffer, the eggs were dehydrated, cleared in toluene and embedded in Araldite; all processes being performed at room temperature.

(2) The method of Nagai & Rebhun (1966). Fixation, rinsing and postosmification were done in potassium acetate buffer containing 7 mM-CaCl₂. Cells were immersed in 2% glutaraldehyde in 0.05 M buffer for 110 min and then washed with two 15 min changes of 0.05 M buffer. Specimens were postosmified in 1% osmium tetroxide in 0.05 M buffer pH 6.1 for 45 min then dehydrated,
cleared and embedded as described. All stages were performed at room temperature.

(3) Karnovsky's method, modified by P. Gould (personal communication). Formalin–glutaraldehyde fixative was prepared by adding 4-5 ml 36% AR formalin to 4 ml of 50% glutaraldehyde and 18 ml 0-2 M sodium cacodylate buffer containing 25 mg of CaCl₂ (anhydrous), the pH being adjusted to 7-4 and the volume brought to 50 ml with distilled water. After 15–20 h fixation at 4 °C specimens were washed for 4 h in several changes of 0-1 M cacodylate buffer and postosmified in 1 % osmium tetroxide buffered with 0-1 M cacodylate and finally washed in 0-1 M buffer. Dehydration was performed at room temperature and the material was embedded in Araldite. Sections obtained by the three methods were stained with lead citrate or uranyl acetate and examined in A.E.I. EM 6 electron microscope, in conjunction with Mr D. Fyfe.

RESULTS

Contraction and relaxation

About a minute after the local application of ca. 1·0 μl of 0·1 % polylysine, M 50000 (2 × 10⁻⁵ M) pH 4·5–7·5 to a region of the animal hemisphere of an undivided fertilized Xenopus egg removed from the vitelline membrane, a striking series of events is seen. The treated area assumes a blackish mottled appearance due to the irregular aggregation of cytoplasmic pigment granules; each focus of aggregation increases in intensity of blackening and the foci of aggregation coalesce quite suddenly to a dense spot, from which macroscopic surface folds apparently due to radial tension can be seen at the egg surface. The complete contractile sequence occupies between 3 and 5 min. After contraction has occurred to its maximum extent, some degree of spontaneous relaxation occurs. There is a gradual disappearance of the lines representing radial tension, and sometimes a tendency for pigment granule redispersal is seen. Judging by the lines radiating from the treated region, polylysine has a similar effect on the vegetal hemisphere, though the sparsity of pigmentation makes the response less easy to observe.

An identical contractile response was elicited with similar concentrations of polylysine of M 2600 and 150000, poly-L-ornithine of M 90000 and histone sulphate. Multicellular embryos also responded though the effect seemed to decrease with increasing cellularization; later blastulae showing only scattered black pigment clusters without massive contraction, reflecting a diminishing magnitude of response with decreasing free surface area/cell. Unfertilized eggs obtained from unpaired females gave erratic results and although the reason for the variability has not been investigated in detail, the age of the egg is probably important; some process of ageing distinct from fertilization may be the decisive factor determining responsiveness. Fertile eggs which have rotated inside the vitelline membrane nearly always gave a contractile response and subsequently divided.
Total immersion of undivided fertilized eggs in 0.1% polylysine of $M_50000$ resulted in mottling of the entire animal hemisphere due to local pigment granule accumulations, followed shortly by lysis in the vegetal region and contraction of the entire egg membrane and underlying pigmented cortical layer to the animal pole.

It was not possible to influence the cleavage of dividing eggs or the direction of subsequent cleavages by means of polylysine applied locally, apart from the purely mechanical effects of tension created at the egg surface due to local induced contractions.

Localized treatment with one particular batch of ribonuclease, $10^{-3} \text{ M}$ at pH 6.5, caused a smooth and extensive contractile response otherwise very similar to that caused by polylysine. This was observed repeatedly, but was not given by other samples of the protein. Partial dimerization of ribonuclease may be related to its effectiveness, but the problem has not been resolved. Cytochrome-c caused no contraction at pH 7.5 or pH 4 where it is known to bind to the surface of Amoeba proteus and induce pinocytosis (Schumaker, 1958). Unpolymerized amino acids gave no response, nor did the polyanions heparin sulphate and sodium polyglutamate.

When eggs in any stage of localized contractile response to polylysine were immersed in 0.1% sodium polyglutamate the lines of tension around the area of contraction disappeared and the pigment granules redispersed, a process which appeared almost exactly like contraction in reverse, the only difference being that pigment was not evenly redistributed; areas of heaviest polylysine-induced pigment concentration relaxed excessively, resulting in sparsely pigmented patches. Immersion of whole untreated eggs in 0.1% sodium polyglutamate had no observable effect; during 2 h at this concentration division proceeded normally, while subsequent local polylysine treatment after removal of polyglutamate resulted in a characteristic contractile response in normal Steinberg's medium. 0.1% heparin at pH 7.0 also reversed the effect of polylysine. When eggs which had been caused to relax in heparin or polyglutamate after polylysine treatment were removed to Steinberg's medium the contractile response was again manifested even after 30 min in polyanion.

The addition of polylysine to eggs in Steinberg's medium containing additional 1.0 M > NaCl > 0.1 M results in normal contraction, showing that fairly high extracellular ionic strength does not inhibit the action of polylysine. However, under these conditions, but in the absence of divalent cations, contraction cannot proceed. Following the addition of divalent cations Ca$^{2+}$ and Mg$^{2+}$ as in normal Steinberg's solution contraction is observed to continue. This shows that certain divalent cations are essential for contraction. Treatment with polylysine in 2.0 M > NaCl > 1.0 M in distilled water or subsequent immersion in 2 M-NaCl after treatment in normal Steinberg's solution prevents contraction. On replacement of the medium by normal Steinberg's solution no initiation of contraction occurs, but the region previously treated with polylysine is seen to be
Contractile response of egg surface capable of contractile response to a new application of the polycation. This suggests that high NaCl concentration elutes surface-bound polylysine, but that lower concentrations do not.

In view of the contractile responses of the cell surface to polycations, the action of ionic detergents was investigated to see if they would have the same effect as polyelectrolytes by virtue of their charge and strong adsorptive characteristics: furthermore, the opportunity of making the cell surface more negative by means of anionic detergent arises. Both cationic and anionic detergents used were of short hydrocarbon chain length (C₁₂–C₁₆). It was found that low concentrations of the detergents elicited a contractile response of the cell surface closely paralleling that caused by polylysine. Hexadecyl trimethylammonium bromide, which bears one positive charge/molecule, at 0.5 mM and sodium dodecyl sulphate which has one negative charge/molecule, at 1.0 mM, applied locally to the animal pole (Fig. 1) cause a very smooth regular aggregation of pigment as the surface contracts, completion of the process taking about 3 min. Higher concentrations of ionic detergents cause lysis of the egg surface. The only apparent distinction between these responses and that due to polylysine is in the smoothness of pigment aggregation following detergent treatment, indicating perhaps a more even adsorption of the smaller molecules. The non-ionic detergents, Pluronic F68 and Triton X-100, were completely without effect on egg surfaces at any stage, even at concentrations of 1%, indicating the importance of the charged group carried by ionic detergents in initiating contraction of the egg surface.

A brief series of experiments on fertilized Amblystoma eggs confirmed results obtained with Xenopus: polylysine and ionic detergents elicited a similar reaction whereas non-ionic detergents had no visible effect. Very rapid spontaneous relaxation was seen.

Extracellular calcium requirement

The experiments which have been described, considered in relation to the surface activity of polyelectrolytes (see references to Katchalsky et al. 1959; Nevo et al. 1955) and detergents, suggested that one explanation for the contractile response might be that these substances adsorb to the membrane surface, cause a change in membrane permeability either by damaging or re-organizing the membrane and that the ensuing ionic fluxes trigger a contractile mechanism beneath the plasma membrane. If this were so, prevention of the entry of divalent ions, which critically determine the state of contraction or relaxation of muscle proteins (Hasselbach, 1969) should inhibit the contractile response to polycation and ionic detergents. In the presence of 3 mM-EDTA, which chelates divalent cations, dissolved in divalent cation-free Steinberg’s medium, neither polycation nor ionic detergents were ever found to elicit the contractile response. If, however, after polylysine treatment in EDTA medium the latter was replaced with Steinberg’s medium containing calcium, contraction started immediately and
Contractile response of egg surface

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proceeded normally. Magnesium at concentrations between 0·1 and 10 mM in Ca\(^{2+}\) free Steinberg's medium did not permit contraction. In order to test whether arrest of contraction was due not to removal of divalent ions but to an inhibitory action of EDTA, the experiment was repeated using ion-free distilled water containing sucrose of the same osmolarity as normal Steinberg's medium. NaCl was not used because of trace amounts of divalent cations present. The results of this experiment were unambiguous with polylysine: contraction never occurred.

Permeability changes

An attempt was made to investigate enhanced permeability to molecules larger than inorganic ions in the regions of the egg surface which had been treated with polylysine or detergent, using dyes. Undivided or divided fertilized eggs were exposed for about 1 min to 0·1 % solutions of the cationic dyes Nile blue sulphate or neutral red, which were introduced in the immediate vicinity of the egg by means of a fine pipette and removed similarly during the course of contraction. After the completion of contraction, and fixation in formaldehyde (which was sometimes omitted), eggs were torn open with fine tungsten needles and examined under a binocular microscope for cytoplasmic staining. When eggs were exposed to the dyes immediately after treatment with polylysine, anionic or cationic detergent before contraction began, the treated surface area appeared strongly stained. Eggs then allowed to undergo contraction in the presence of external dye show intense staining at the contracted regions after removal from the dye, and a thin layer of cortical cytoplasm also appeared to be stained: when an egg thus treated was punctured, stained cortical cytoplasm was extruded as normal healing proceeded, but it is possible that dye was picked up from the surface as cytoplasm passed out.

These preliminary studies were sufficiently suggestive of membrane permeability changes in response to superficial adsorption of polycation to warrant investigation using intracellular microelectrodes (Gingell & Palmer, 1968). It was found that the cell membrane resistance fell as much as tenfold when polylysine was applied locally. In the absence of extracellular Ca\(^{2+}\) the fall in resistance still occurred but no contraction occurred until 0·34 mM-Ca\(^{2+}\) was replenished. Polyglutamate alone caused no electrical response.

Fig. 1. Successive stages of the contractile response of a fertilized Xenopus laevis egg to 1 mM sodium dodecyl sulphate applied locally to the animal pole are shown. The sequence runs left to right and top to bottom. The first frame depicts the untreated egg, the second was taken immediately after treatment. Subsequent frames are separated by ca. 1 min intervals. During contraction, the egg also divided, but with the exception of the thin vertical cleavage line in the latter four frames and actual division in the last, the appearance is typical of the action of agents which cause contraction.

The essential feature is the rapid development of a dark area (upper centre, frame 4) which then seems to coalesce to a small dense black spot (upper centre, frame 5). × 30 approx.
During a series of experiments ribonuclease was found to cause contraction and lowering of resistance followed within a few minutes by relaxation and restoration of resistance, but unfortunately this was unrepeatable with other samples of the enzyme, suggesting possibly that a strongly cationic impurity in the original sample was the active factor.

These results confirm that membrane permeability to small ions is increased as a result of the adsorption of polycations.

**Surface changes during cell division**

During the dye experiments, an unexpected and interesting observation was made in connexion with the cleavage furrows of early blastulae. Exposure of treated or untreated dividing eggs to 0.1% Nile blue sulphate for 30 sec or 1 min, followed by 1 or 2 min washing in Steinberg's medium, resulted in localized dye accumulation in the region of the cleavage furrow. In the early stages of division, the dye stained a discrete unpigmented region either side of the cleavage furrow on the animal hemisphere, but there was no trace of staining actually in the cleavage furrow or anywhere else on the egg surface, though it was not easy to decide whether the densely pigmented contractile band which borders the stainable unpigmented furrow region had an affinity for the dye. As division proceeded, the previously stained bands on either side of the cleavage furrow came to occupy progressively less surface area. This also indicates local contraction. When eggs were stained near the end of the cytoplasmic division when the blastomeres were practically parted, a broad stained unpigmented band was seen running completely around the blastomeres on either side of the white isthmus which still joined them. This area corresponds to the newly synthesized surface described by Selman & Waddington (1955). It is surprising that no other dyes tried, including the cationic dyes methylene blue and toluidine blue which have an amino-quinone ring structure like Nile blue sulphate, nor alcian blue, had any effect at all when used under similar conditions. Similarly, the anionic dyes eosin B, orange C and chorazol black were ineffective in staining the cleavage furrow region.

Apparent tension, indicated by radiating stress lines in the egg surface, suggests that at least the onset of cytoplasmic division is dependent on a contractile process which may be identical to that responsible for contraction in response to polycations and ionic detergents. The apparent reduction in surface area, shown by the Nile blue sulphate staining reaction described, strengthens this interpretation.

**Wound healing**

When a small puncture wound is made in the cell surface with a fine tungsten wire, in the presence of extracellular 0.34 mM-Ca\(^{2+}\), a contractile wound response can be observed. The edges of the wound blacken due to localized pigment granule aggregation and radial lines of tension are set up. It is evident that
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elements at the wound perimeter are contracting since the perimeter of the wound progressively decreases, and whatever the original shape of the wound, it tends to become circular as closure proceeds. Finally, a small black dot of pigment granules, which slowly disperse, marks the closed wound. Larger wounds close in a similar fashion, often with the loss of a high proportion of the total cytoplasm.

The divalent cation requirement for wound healing was investigated using Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\) made up in otherwise divalent cation-free Steinberg’s medium. Magnesium chloride between 0.1 and 10.0 mM does not allow wound healing to occur, in distinction to the other salts which act in this range. Calcium is most active, allowing normal wound healing to occur at 0.1 mM but not at 0.01 mM or at over 30 mM. In the latter concentration closure begins but rapidly reverses, presumably as the calcium concentration in the cytoplasm adjacent to the wound margin approaches that of the external solution. SrSO\(_4\) and BaCl\(_2\) are able to substitute for Ca\(^{2+}\) at 1.0 mM but are inactive at 0.1 mM. Wounds closing in 1.0 mM–Ba\(^{2+}\) or Sr\(^{2+}\) relax in 0.1 mM–Ba\(^{2+}\) or Sr\(^{2+}\) and recontract in 1.0 mM concentrations of these salts. The relatively high, lower active concentration of Ca\(^{2+}\) > 0.01 mM precludes the possibility that Ca\(^{2+}\) contamination of the other salts used could be the basis of their activity.

The salts were also tested for their ability to promote contraction in response to polylysine treatment, and the results paralleled those obtained for wound healing.

Electron microscopy

(i) Untreated eggs (Fig. 3A, B, C). Electron microscopy of fertilized untreated eggs prior to first cleavage shows an asymmetric plasma membrane about 80 Å thick, which sometimes appears to be bounded on the outer side by an extracellular coat of low but variable electron density, 100–300 Å thick. None of the fixation methods employed, however, gave satisfactory preservation of untreated cell surface regions. Beneath the membrane is a zone abounding in vesicles (see Figs. 8, 10) often apparently lined with extracellular coat material, suggesting their pinocytotic origin. The zone immediately beneath the plasma membrane but external to the pigment granules apparently corresponds to the hypolemma of Dollander (1960, 1962). It contains a profusion of granules, possibly glycogen as well as ribosomes, and occasional suggestions of a filamentous material, though fibres have not been satisfactorily visualized after any of the fixation methods employed in this study. Pigment granules and other large bodies are excluded, as if kept out by a spongy network. Below is a region characterized by pigment granules (Figs. 2, 3) profuse at the animal pole, sometimes seen to be enclosed in a membrane. Vesicles, ‘lipid’ droplets (perhaps a protein-lipid complex remaining after elution of free lipid by the preparative solvents: these inclusions appear to be the ‘lipochondries’ of Van Gansen, 1966a, b), mitochondria and yolk platelets, constitute the bulk of the cytoplasmic
inclusions. Pigment granules are much more sparsely scattered in the vegetal region where large yolk platelets predominate.

(ii) *After polylysine treatment* (Figs. 2, 4-6). Following the localized application of polylysine to the animal pole of fertilized eggs, local peripheral organization of the cell is greatly changed: the plasma membrane and surface coat are thrown into extensive folds reminiscent of microvilli and outside the plasma
membrane there is a thick electron-dense layer (Fig. 6) which almost certainly results from adsorption of the polymer to the cell surface. The normally thin underlying granular hypolemma seen in untreated cells is considerably increased in radial thickness, with the characteristic exclusion of larger bodies. Pigment, lipid droplets, mitochondria and yolk platelets are absent. As in untreated cells, there are sometimes traces suggestive of fibrils in this layer, but they have not been identified with certainty. At low magnification, the zone appears as an electron-dense band, more or less continuous, but of lower density in the surface folds. Vesicles are sometimes found in the surface folds as well as in the dense band; when they occur in the latter, they are often flattened and oriented parallel to the cell surface. Beneath this layer is a massive aggregation of pigment granules, responsible for the macroscopic blackening of the contracted region. Other cytoplasmic inclusions are similarly condensed at a considerable depth.

Fig. 4 in addition shows relatively electron-transparent areas with bodies which bear a marked resemblance to the 'plages des polyribosomes' described by Van Gansen (1966a, b) in fertilized *Xenopus laevis* eggs, though Hay (1966) has shown how glycogen can mimic polyribosomes in *Xenopus* embryos. These areas have not been examined at higher power.

Measurements made on selected sections cut perpendicularly to the surface, show that the plasma membrane is of similar dimensions, around 80 Å thick, in both treated and untreated regions of fertilized and unfertilized eggs in single cell and early blastula stages. It has been noticed that the plasma membrane of polylysine-treated regions is frequently more symmetrical than untreated surface membrane, showing two electron-dense lines of about equal density and thickness, and apparently in a better state of preservation, but whether the different appearance is due to structural modification of the membrane by

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Fig. 2. View at low magnification of a region of the animal pole of a fertilized egg treated locally with polylysine. The extremity of the treated region is recognizable at the left-hand side by superficial folding and pigment granule accumulation in the cytoplasm. The right-hand side has not been treated and typifies the uncontracted surface. c = Thickened submembranous region where polylysine was added. Surface thrown into pronounced folds; h = submembranous layer or hypolemma thickened and more electron dense near treated region; l = probably lipid; m = mitochondrion; p = pigment granule; y = yolk. x 1000.

Fig. 3 A, B. Sections of animal pole of untreated fertilized egg. Compare well-dispersed pigment granules (p) with unevenly distributed granules in Fig. 2 which are concentrated beneath the treated region of the cell surface. Hypolemma (h) is of similar electron density to deeper matrix. a = Artifact due to pigment loss from section; v = vesicle. Other components labelled as indicated in Fig. 2. (A) x 4000 approx. (B) x 7500.

Fig. 3C. Section of animal pole of untreated fertilized egg. The plasma membrane (pm) is apparently composed of a densely staining inner lamina and a weakly staining outer lamina. Very faint traces of material external to the outer lamina may represent a superficial coat. Granules (g), probably glycogen or ribosomes, abound in a matrix of irregular electron density. x 200000.
polylsine as seen in pinocytosing amoebae by Brandt & Freeman (1967) or to a surface layer of polylsine affording some degree of protection to the underlying plasma membrane during the rigours of fixation, dehydration and embedding is not known. It is certainly not impossible that the extra-membranous material of untreated eggs which has been equated with 'surface coat' is merely a product of the degeneration of the outer dense line of the plasma membrane. During this study no really satisfactory high-power pictures of the untreated cell membrane were made: all had an indistinctness at the outer extremity (e.g. Fig. 3C). Although breaks in the plasma membrane do sometimes occur in the later stages of vigorous polylsine-induced contractions, possibly as a result of mechanical stress, there is no clear evidence that rupture of the membrane precedes contraction\(^1\) (p. 606). Breakage is shown by the appearance of cytoplasmic inclusions outside the surface, and should not be confused with loss of definition of the surface membrane where it is tangential to the plane of section. This is seen in Fig. 5 where the valleys of the surface folds show indistinct surface membrane due to tangential sectioning. It is also true of Fig. 4 though the effect is not so noticeable at lower magnification.

Eggs in early cleavage stages treated locally with polylsine, allowed to contract and then relaxed in the presence of 0·1 % polyglutamate (Figs. 7, 8) show unevenly redistributed pigment granules and an extracellular layer of greater density than that present on untreated cells, and it is probable that some polylsine is still absorbed at the surface, but there is no evidence of surface folding associated with the contracted state. No discontinuities suggestive of lysis are seen in the plasma membrane, though the possibility that they occur rapidly and are spontaneously repaired cannot be excluded (Seeman, 1967).

(iii) Spontaneous repair of a wound. A wound made with a fine tungsten needle in the egg cell membrane was allowed to close by contraction in normal Steinberg's medium for a minute before fixation. Examination at low power (Fig. 9) shows a striking resemblance to polycation-induced contraction. Adjacent to the wound margin the surface is thrown into deep folds apparently by contraction of a layer immediately beneath it. This thickened region usually excludes larger bodies such as pigment granules, suggesting that it constitutes a mechanical barrier.

At higher power (Fig. 10) the region immediately beneath the membrane has

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Fig. 4. Animal pole contracted after localized treatment with polylsine. The surface is thrown into microvillus-like protrusions, the region (c) separating the pigment granules and the cell surface has become considerably more electron dense and has thickened and all cellular inclusions are closely aggregated. Many areas (r) can be seen which may possibly correspond to the 'plages des ribosomes' described by Van Gansen (1966a, b). A pigment granule (p) is apparently inside one of these areas. The section is slightly tangential. \( \times 9000 \).  

Fig. 5. Contracted animal pole of a fertilized egg following polylsine treatment. Material present outside the plasma membrane is clearly visible. The section is slightly tangential. Artifacts (a) due to sectioning cell surface at grazing incidence are present. \( \times 40000 \).
Fig. 6. High power view of the contracted cell surface after treatment with polylysine. Abundant material adsorbed to the outside of the plasma membrane (pm) can be seen. Glycogen granules or ribosomes (g) are common. × 316000.

Fig. 7. Section through the animal pole of a fertilized egg which was caused to contract locally by treatment at the animal pole with polylysine, and subsequently completely relaxed in the presence of sodium polyglutamate. Surface protrusions have disappeared but the pigment granules have not redistributed uniformly (cf. Fig. 3). Other components labelled as indicated in Fig. 2. v = vesicle. × 10000.

Fig. 8. The same section as shown in the previous figure. Material apparently still adsorbed at the outer surface of the plasma membrane is just visible. In this and the previous figure note that pigment rarely contacts the plasma membrane. Vesicles (v) reminiscent of pinocytotic vesicles, which clearly contain surface material are abundant. g as in Fig. 6, other components labelled as indicated in Fig. 2. × 52000.
Fig. 9. Edge of closing wound (w) in the animal pole of a fertilized egg. The ruptured region can be seen extruding yolk and pigment granules. Surface convolutions due to submembranous thickening (c) can be seen to one side of the wound. Loss of pigment granules from the section had given rise to artifacts (a). ×1800.
Fig. 10. Surface region adjacent to the wound, showing a few traces of fibrillar organization ($f$). Vesicles ($v$) are present. Blurring of the cell surface due to grazing incidence sectioning in certain areas ($a$) gives a false impression of damage. $\times 138000$. 
the appearance of a spongy network of low electron density, with vesicles and granules present. Good resolution of fibrillar material has not been achieved and the cell membrane is poorly preserved. It is, nevertheless, clear that the dense extramembranous layer seen after polylysine treatment is not present.

In common with polycation-induced contraction, wound closure contraction is operative in the presence of extracellular Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\) but not Mg\(^{2+}\) alone. 1 mM-EDTA in divalent cation-free Steinberg's medium inhibits closure.

**DISCUSSION**

*Adsorption of basic polyamino acids and ionic detergents*

If the surface of *Xenopus* eggs bears a preponderance of fixed negative charges in common with other cell surfaces, it would be expected that positively charged macromolecules would be readily adsorbed at the cell surface. The adsorption of cationic polyamino acids to cell surfaces has been extensively investigated in Katchalsky's laboratory; Nevo, de Vries & Katchalsky (1955) demonstrated electrophoretically that polylysine HBr binds to the erythrocyte membrane without penetrating into the cell interior. Electron microscopy of agglutinated erythrocytes by Katchalsky, Danon & Nevo (1959) showed surface-bound material bridging the gap between adjacent cells, the bridge length corresponding closely with the length of fully extended polymer molecules. The dense layer present at the cell surface outside the plasma membrane of the *Xenopus* egg after polylysine treatment almost certainly represents adsorbed polycation, whose orientation cannot be perpendicular to the surface. The length of a fully extended polylysine chain of $M = 5 \times 10^4$ is ca. $1.5 \times 10^3$ Å while the electron-dense layer is around $3 \times 10^2$ Å thick after polylysine treatment regardless of whether $M$ of $2.6 \times 10^2$, $5 \times 10^4$ or $15 \times 10^4$ were used. Therefore, the polycation chains are probably folded and attached to surface carboxyl groups at multiple points. Danon, Howe & Lee (1965) have described the interaction of polylysine with the erythrocyte membrane and suggested that the polybase interacts with N-acetyl neuraminic acid groups of a superficial glycoprotein. Treatment of *Amoeba proteus* with fluorescein-labelled polylysine (Gingell, 1967a) has shown that polylysine of $M = 5 \times 10^4$ adsorbs at the cell surface and that it is not removed by 0.2 m-NaCl or 0.1% sodium polyglutamate of $M = 8 \times 10^4$ at pH 7.0. The strength of superficial polycation binding is emphasized by the apparent inability of polyglutamate or 0.2 m-NaCl to elute polylysine which has absorbed, but its ionic nature is shown by removal of polylysine in the presence of 2 m-NaCl. These facts, in conjunction with the appearance of an electron-dense layer immediately outside the plasma membrane after polylysine treatment, support the view that polylysine binds ionically to the outer surface of the plasma membrane, perhaps to a superficial coat like that of *Amoeba proteus* which may be present at the surface of *Xenopus laevis* eggs.

Although adsorption of surface active agents at the cell surface has not been
directly demonstrated, it is likely that this is their primary site of action. Adsorption of ionic detergent molecules from a polar base phase to a non-polar phase with interfacial fixed charges would proceed by orientation of the hydrocarbon chains and their insertion into the non-polar phase, leaving the polar groups near the plane of the interface. The total free energy change or work done on adsorption of an ionic detergent molecule includes an electrical term (neglecting image forces) and a non-electrical term which represents the free energy of transference of the hydrocarbon chain from water to lipid. The electrical term depends on the signs of the ionic head group charge of the detergent molecule and the sign and magnitude of the surface potential. Since the hydrocarbon chain lengths of the hexadecyl trimethylammonium ion (C\textsubscript{16}) and dodecyl sulphate ion (C\textsubscript{12}) are similar, differences in the rate of adsorption at a charged interface will depend almost entirely on the electrical free energy terms: adsorption of positive ions at a negatively charged interface will be favoured by a reduction in free energy whereas the converse is true in the case of negative ions adsorbing at a negative interface. Thus, hexadecyl trimethylammonium ions would adsorb more readily than dodecyl sulphate ions at a negatively charged cell surface, which is in accordance with the observation that a lower molarity of the positively charged ion is required to trigger the contractile response.

*The site of contraction*

There are four broadly definable regions in which the mechanical elements of the mechanism responsible for the contractile phenomena of response to poly­cations, ionic detergents, wound healing and cell division might reside. These are the ‘extramembranous coat’, the plasma membrane, the hypolemma (outermost region of the cytoplasm) or a deeper region in the cytoplasm. Although it is most unlikely that contraction of the membrane or extramembranous coat would result in their being thrown into folds, which are more suggestive of passive inelastic accommodation to the contraction of a submembranous region, the possibility cannot be disregarded in view of the contractile superficial coat postulated by Holtfreter (1943a, b; 1944, 1946, 1947).

Although in his earlier papers Holtfreter regarded the surface coat as the source of contractility in wound healing and in cell shape changes seen in invagi­nation of the archenteron during gastrulation, he later conceded the possibility that both plasma membrane and perhaps adhering ‘plasmagel’ might be responsible. In concluding that cell division in amphibian eggs involves a superficial contractile mechanism (Holtfreter, 1947) he repeated his uncertainty about the exact site of the force responsible. Nevertheless, Holtfreter continued to argue that amoeboid movement in isolated amphibian cells is due to ‘periodic expansions and contractions of certain areas of the cell membrane’ (Holtfreter, 1947).

There is some electron microscopic evidence for some sort of extramembranous layer in amphibian eggs and early cleavage stage cells, which according to Wartenberg & Schmidt (1961) is derived from cortical granules expelled into
Contractile response of egg surface

the perivitelline space soon after fertilization, the material of which lines the inside of the vitelline membrane and the outer surface of the plasma membrane. Its electron microscopic appearance has been described by Perry & Waddington (1966) and also by Dollander (1962) who terms it the ‘superstructure exovulaire’. Superficial material is discernible in electron micrographs of Baker (1965). The relationship between this structure and the superficial coat of Holtfreter has been discussed by Dollander (1962) who concludes that the extramembranous coat corresponds morphologically but not physiologically with that postulated by Holtfreter since it seems to lack contractile properties.

Contraction of the plasma membrane itself, or perhaps an extramembranous region is an arguable possibility, especially since evidence that nuclear membranes can contract reversibly in response to changes in ionic environment has been provided by Korohoda et al. (1968). Following Kuhn, Ramel & Walters (1960) these authors argue that adsorption of extended charged polyions of opposite sign to the surface would partially neutralize the surface fixed charges and that contraction, due to Van der Waals–London attractive forces between atoms of surface macromolecules, would consequently occur. However, there are two main features which effectively preclude this kind of explanation in the contraction described in *Xenopus*. Firstly, there is a structural consideration which is difficult to reconcile with a membranous or extramembranous contractile zone. Isodiametric contraction of the surface coat or plasma membrane while they remain in mutual contact could hardly result in multiple surface folding. Anisodiametric contraction might lead to folding, but the electron-microscopic appearance of the plasma membrane gives no hint of this. Secondly, the mechano-chemical mechanism outlined would predict contraction at high ionic strength (a point omitted by Korohoda et al. 1968). From the results presented it is clear that the effect of increasing the ionic strength is not what would be expected if direct mechano-chemical contraction of macromolecules at the surface were responsible for contraction. Furthermore, this mechano-chemical mechanism cannot easily explain the similar action of cationic and anionic detergents. Induced contraction by charge neutralization of surface protein, mucopolysaccharide or even the adsorbing macromolecules themselves, as the source of contractile energy, is therefore considered highly improbable.

The most likely location for the contractile elements seems to be in the hypolemma. Increased thickness of the zone following polylysine treatment of the cell surface, and the appearance of fibrillar organization is in keeping with the concept of a contractile region. The more electron-dense material seldom invades the surface folds, which remain populated by granules, sieved perhaps through a fibrillar network by increasing hydrostatic pressure as contraction ensues; an interpretation which is supported by the exclusion of larger bodies from the folds. Multiple folding of the surface rather than the formation of one large bulge might be due to the attachment of fibrils to the underside of the plasma membrane. Comparison of Figs. 3 and 4 emphasizes a feature which is
difficult to explain unless the region beneath the membrane and external to the pigment granules contracts anisodiametrically. The question is simply, how can the radial thickness of the pigment granule-free layer increase unless it is contracting actively? Neither contraction of the plasma membrane, an outer coat, nor deeper cytoplasmic regions would be expected to thicken the hypolemma. Thickening must almost certainly be due to contraction of this layer in a direction parallel to the surface. If it tended to contract equally in all directions, it would not thicken in any one direction. The fact that vesicles found in this layer are occasionally round suggests at first sight that they are subject to equal deforming tensions in all directions, but if they are not freely permeable asymmetric forces would tend to translocate but not deform them. The evidence from electron microscopy therefore suggests strongly that contraction takes place in the cytoplasm beneath the cell surface membrane.

**Iontophoretic injection of Ca\(^{2+}\)**

Ca\(^{2+}\) appears to interact with the contractile system, to which it gains entry from the extracellular environment by virtue of the lowered membrane resistance caused by adsorbed ionic molecules (Gingell & Palmer, 1968). Direct access to Ca\(^{2+}\) follows mechanical wounding of the membrane, resulting in exactly the same contractile response.

A critical test of this hypothesis was performed by iontophoretic injection via a glass microelectrode. Contraction could be induced by passing Ca\(^{2+}\) ions into the region immediately beneath the cell membrane, but not when the electrode tip was positioned deeper in the cytoplasm or just outside the cell surface. The passage of current at \(3 \times 10^{-8}\) A in square pulses of 200 msec duration for 1 min at a frequency of 5 pulses/sec. from a Grass stimulator by a microelectrode containing 1·0 mM-CaCl\(_2\) was sufficient to cause noticeable contraction around the electrode tip. Thus a localized Ca\(^{2+}\) ion influx of the order of \(10^9\) ions can initiate visible contraction. No response was observed following prolonged injection of Mg\(^{2+}\), K\(^+\), Na\(^+\), Cl\(^-\). Other divalent cations which have been shown to allow wound closure and polycation-induced contraction have not yet been investigated, but these results confirm the experiments in which Mg\(^{2+}\) was unable to substitute for Ca\(^{2+}\) in contractile processes.

After cessation of the current flow, relaxation of the previously contracted zone proceeded spontaneously within a minute or two. Simple diffusion away from the region of injection or possibly active sequestering of Ca\(^{2+}\) could account for relaxation.

**The cortical contractile zone in embryonic cells: a common feature?**

The electron microscopic evidence from cells treated with ions which increase membrane permeability to Ca\(^{2+}\) and from mechanical wounding, taken in conjunction with iontophoretic injection of Ca\(^{2+}\) show that the region responsible for contraction lies just beneath the cell surface membrane. There is no
Contractile response of egg surface

Evidence that the cell membrane or surface coat, if present, contribute to contraction. The classic wound-healing response can be explained as a cytoplasmic phenomenon, confirming the suggestion put forward by Dollander (1960).

It seems likely that the contractile properties of the cortical region which have been demonstrated are of primary importance in a wide variety of morphogenetic movements, and the presence of such a region has been postulated and adduced from electron micrographs for some time. Wolpert (1963) described the appearance of an apparently contractile zone beneath sea urchin egg membrane, which seems to be involved in cytoplasmic division. Baker (1965) gives evidence for the contractility of a similar layer beneath the plasma membrane of invaginating archenteron cells in gastrulating Hyla embryos. The layer becomes progressively thicker in cuboid, wedge and flask cells, with folding of the surface membrane, narrowing of the cell neck and decrease in the projected area of the free cell surface (cf. also Trinkhaus & Lentz, 1967). A similar dense layer is recognized by Perry & Waddington (1966) in flask cells of Triturus gastrulae, though these authors attribute change in cell shape to active elongation associated with longitudinal microtubules. However, the stellate cross-section of flask cell necks seems at least equally explicable if contraction of the dense zone extending down the cell necks throws the lateral walls into folds like those of the free surface and forces the cell to elongate by cytoplasmic displacement. A dense layer, 0.08-0.2 μ thick, was found by Balinsky (1961) beneath the neural plate cell membranes of anuran embryos, while cytoplasmic filaments orientated parallel to the highly convoluted free cell surface have recently been found in invaginating amphibian neural tube cells by Baker & Schroeder (1967). These authors conclude that contraction of the dense layer is probably responsible for the wedge-shape of the cells. A localized wrinkling reaction of sturgeon eggs in response to stroking a glass needle across the outer or inner surfaces of the cell membrane has been described by Zotin (1964), who expressed his opinion that the contractile mechanism responsible lies in the peripheral cytoplasm and is the same as that which participates in cytoplasmic division. In larval ascidian epidermis, Cloney (1966) clearly showed the presence of apparently contractile filaments, immediately beneath the plasma membrane, which shorten by a factor of about 70 during tail resorption.

It can therefore be seen that a cortical contractile zone is a feature not only of amphibian eggs, but is almost certainly present in numerous embryonic cell types where it provides the motile force for morphogenetic movements. The possibility that a similar motile mechanism may be involved in the movement and adhesion of adult cells is attractive (Gingell & Garrod, 1969; Gingell, Garrod & Palmer, 1969).

It has been shown that operation of the cortical contractile mechanism can be externally triggered by an increase in membrane permeability which admits Ca^{2+} in response to polycation and ionic detergent adsorption at the cell surface. But what is the sequence of events in spontaneous contraction occurring in
cleavage and morphogenetic changes? Does cell membrane permeability increase locally as a result of the interaction of cytoplasmic triggering molecules with the inside of the cell membrane? Although the rather crude dye experiments suggest that such regions of the surface are different in some respects from the rest of the cell surface, the observation that cell division proceeds in *Xenopus* blastulae removed from the vitelline membrane in Steinberg’s medium lacking divalent cations and with added 1 mM EDTA (C. Slack, personal communication) argues against the entry of Ca$^{2+}$. However, it is conceivable that the changing ionic fluxes through the cell surfaces which must presumably follow the irregular fall in blastula cell membrane resistance (Ito & Hori, 1966; Palmer & Slack, 1969) might initiate release of Ca$^{2+}$ previously bound in an inactive state in the region of the contractile zone. Alternatively, release of such Ca$^{2+}$ might be the result of an entirely internal triggering event.

None of these mechanisms bear on the problem of the localization of contraction at discrete regions of the surface. In the case of cell division, it is known that the plane of cytoplasmic cleavage is determined with respect to the co-ordinates of nuclear division and so it is not difficult to conceive of an activating reaction causing Ca$^{2+}$ release near the surface in a pattern geometrically specified by the mitotic apparatus.

There is a more severe difficulty in interpreting unilateral contraction seen for example in invaginating archenteron or neurula cells, and it is not difficult to understand why a ‘contractile surface coat’ was an attractive hypothesis since it automatically provided anisodiametricity. It is possible that in cell sheets the contractile zone beneath the membrane is present only on the side forming the outer face of the sheet and that contraction follows a non-directional internal stimulus. The obvious alternative is of course a uniformly distributed sub membranous contractile system activated by directional information from within the cell, but the matter is at present quite obscure.

*A transducer mechanism*

Although the existence of a cytoplasmic contractile mechanism is of great interest in its own right, its activation by the surface binding of strongly charged molecules may be interpreted in terms of a transducer mechanism which might be of wider significance. It has been suggested that changes in cell surface electrostatic potential may be a common mediator between environmental input and ionic output from the membrane transducer (Gingell, 1967b, 1968; Wolpert & Gingell, 1968; Gingell, 1970) in a number of different systems, including the initiation of pinocytosis in large free-living amoebae.

The increase in permeability caused by the apparently non-specific adsorption of strongly charged molecules suggests that the charge carried rather than the configuration of the adsorbed molecule is the factor which determines membrane response. In other words the change in membrane electrostatic surface potential due to charge density change may well be the critical event.
Lack of overriding chemical specificity in the ligand-cell surface interaction responsible for initiating the permeability transformation is clear. While the action of ribonuclease is in question, histone sulphate, poly-L-lysine HBr ($M$ 2600; 50000; 150000), poly-L-ornithine and hexadecyl trimethylammonium bromide are active; all are highly ionized cationic molecules at around neutral pH. Non-ionic species and neutral amino acids are ineffective, as are polyanions. However, the small anion sodium dodecyl sulphate is active, and therefore represents something of a problem.

If the charge carried is responsible for initiating the permeability change then it must be concluded that either an increase in positive or negative charge can be effective. On the other hand this detergent, and perhaps hexadecyl trimethylammonium bromide, may interact hydrophobically with the surface by virtue of their short hydrocarbon chains. Lack of activity of large highly polymeric non-ionic detergents does not necessarily obviate this interpretation.

Unfortunately it is not possible to parallel a resistive effect of a rise in negative surface potential by the obvious method of reducing the concentration of external sodium which is the predominant cation. This is because reducing bulk phase ionic strength increases the ratio Ca$^{2+}$/Na$^+$ near (possibly bound to) the surface, and results in increased membrane resistance (see Luttgau & Niedergerke, 1958). However, on replacing normal medium for divalent cation-free Steinberg's medium with 0.0058 M-NaCl made isotonic with sorbitol, a small rise in resistance still occurs which cannot easily be attributed to increased Ca$^{2+}$/Na$^+$ near the surface. It is possible that this represents a resistive response simply to increased surface negative potential resulting from lowered ionic strength.

If then, the surface must be made positive in order to initiate a permeability increase, it might be anticipated that raising the ionic strength sufficiently might prevent such a permeability increase by lowering the magnitude of the positive potential due to the polycation. Treatment of eggs in Steinberg's medium containing 0.5 M-NaCl with 1% polylysine ($M$ 195000) resulted in normal contraction: in this case the local positive surface potential is calculated to be approximately one-third of that in normal Steinberg's medium, so it is clear that such a reduction in positive potential is insufficient to prevent the permeability increase. But in medium containing NaCl > 1 M where the potential at the surface of a polyon would be reduced by at least a factor of four, electrostatic binding between surface and polyon is prevented and contraction does not occur. Thus it can be said only that increasing ionic strength does not prevent a permeability transformation due to adsorbed polycation below the point at which the reduction in potential prevents ionic binding of the polyon.

It must be concluded that although most of the evidence suggests that a non-specific increase in surface positivity is the common factor linking the action of polycations, the action of ionic detergents is still far from settled.

Nevertheless, it is clear that subsequent changes in membrane organization
result in an increase in permeability to Ca\textsuperscript{2+} and probably also to larger molecules. It is considered unlikely that membrane puncture causes the permeability increase. Cytoplasmic contraction, the overt cellular response, is normally initiated by Ca\textsuperscript{2+} ions interacting reversibly with a cortical contractile system, though alternative operation of the system with Ba\textsuperscript{2+} and Sr\textsuperscript{2+} but not Mg\textsuperscript{2+} shows that it lacks rigid specificity, and resembles the muscular actinomyosin mechanism. Although in the case of pinocytosis in Amoeba the nature of the ionic output from the transducer is unknown, the overall mechanism shares in common with that described for Xenopus, an increase in membrane permeability resulting in cytoplasmic motile response.

\textsuperscript{1} Note added in proof. An observed fall in electrical resistance from 150 kΩ cm\textsuperscript{2} to 30 kΩ cm\textsuperscript{2} might result from an additional parallel resistance of an area of 'membrane' having the specific resistance of cytoplasm—i.e. a hole. The area is found to be < 1 Å\textsuperscript{2}. Such an explanation is therefore quite unrealistic.

**SUMMARY**

1. The discrete application of highly charged polycations, as well as ionic detergents, to the surface of *Xenopus laevis* eggs and early cleavage stage cells after removal of the vitelline membrane elicits a dramatic localized contractile response.

2. The presence of Ca\textsuperscript{2+}, Sr\textsuperscript{2+} or Ba\textsuperscript{2+} in the external solution is essential for contraction but Mg\textsuperscript{2+} is ineffective.

3. Following contraction, the subsequent removal of external calcium with EDTA as well as the addition of polyanions promotes relaxation of the cell surface.

4. It was initially suspected that the cell surface membrane was undergoing contraction and relaxation but electron microscopic examination has revealed that the surface membrane is thrown into extensive folds as a result of contraction in the underlying cytoplasm.

5. The striking morphological similarity with wound closure in *Xenopus* eggs suggests that the same contractile elements are operative in both cases.

6. It is questionable whether polycation and detergent action simply disrupts the surface membrane, or acts in a more subtle way. Electron microscopic and electrophysiological studies of contracting and relaxing surfaces indicate that membrane destruction is not necessarily a prerequisite of contraction.

7. It appears that a change in membrane permeability, triggered by superficial adsorption of charged molecules altering the surface potential, allows an influx of Ca\textsuperscript{2+} ions which interact with a cytoplasmic contractile system. Iontophoretic injection of Ca\textsuperscript{2+} just beneath the cell membrane confirms this conclusion. The change in electrostatic surface potential may thus be envisaged as a transducer transforming environmental information into more specific ionic information to which the cytoplasm is able to respond.
RÉSUMÉ
La réponse contractile de la surface d'œufs

1. L'application discrète de polycations fortement chargés ou de détergents ioniques à la surface d'œufs de *Xenopus laevis* et de cellules d'embryons au début de la segmentation, après enlèvement de la membrane vitelline, provoque une forte réponse contractile localisée.

2. La présence de Ca\(^{2+}\), Sr\(^{2+}\) ou de Ba\(^{2+}\) dans la solution extérieure est essentielle pour que la contraction se produise, mais le Mg\(^{2+}\) est sans effet.

3. Si on enlève, après contraction le calcium extérieur avec de l'EDTA ou si on ajoute des polyansions, on favorise la relaxation de la surface cellulaire.

4. On a supposé d'abord que la membrane superficielle de la cellule subissait des contractions et des relâchements, mais l'examen au microscope électronique a montré que cette membrane forme des replis à la suite de la contraction du cytoplasme sous-jacent.

5. La similitude frappante avec la fermeture d'une blessure chez les œufs de *Xenopus* suggère que les mêmes éléments contractiles interviennent dans les deux cas.

6. On peut se demander si les polycations et les détergents ont simplement pour effet de disloquer la membrane superficielle ou s'ils agissent de manière plus subtile. L'étude au microscope électronique des surfaces en contraction et en relaxation indique que la destruction de la membrane n'est pas nécessaire pour que la contraction se produise.

7. Il semble qu'un changement de perméabilité de la membrane, déclenché par l'adsorption superficielle de molécules chargées modifiant le potentiel de surface, permet un influx d'ions Ca\(^{2+}\) qui réagit avec un système cytoplasmique contractile. L'injection iontophorétique de Ca\(^{2+}\) juste sous la membrane de la cellule confirme cette conclusion. Le changement dans le potentiel électrostatique de surface peut donc être considéré comme un mécanisme permettant la transformation de l'information environnante en information ionique plus spécifique à laquelle le cytoplasme est capable de répondre.

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Contractile response of egg surface


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