

# WAVE OF STIFFNESS PROPAGATING ALONG THE SURFACE OF THE NEWT EGG DURING CLEAVAGE

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## ABSTRACT

In the eggs of the newt, *Cynops (Triturus) pyrrhogaster*, change in stiffness of the cortex was measured in various regions at the time of the cleavage. Measurements were performed by Mitchison and Swann's cell elastimeter method with a modification, in which two fine pipettes were attached to the surface of one egg at the same time, in order to compare the rigidity of two regions.

The stiffness of the cortex changed very little before the start of the first cleavage. However, just before the appearance of the first cleavage furrow, the stiffness increased rapidly at the animal pole region, which later returned to the former level. As the cleavage furrow progressed, a wave of high stiffness travelled meridionally as a belt along the surface from the animal pole region toward the vegetal region. At second cleavage, the cycle of change in stiffness was repeated.

## INTRODUCTION

In the cleaving eggs of the newt, *Cynops (Triturus) pyrrhogaster*, Sawai (9) demonstrated that if the subcortical cytoplasm derived from under the cleavage furrow was transplanted beneath the cortex far distant from the cleavage plane, the 'cleavage furrow' was induced at the region of transplantation. However, the time of the reaction of the cortex toward the transplant (to form a cleavage furrow) shifted in reference to the progress of the furrow of the host eggs. Stated more fully, with the beginning of the first cleavage, the reactivity of the cortex to the transplanted cytoplasm could only be detected in the animal pole region, and the reactivity traveled down meridionally toward the vegetal pole as the cleavage furrow of the host eggs progressed. A priori one might think that such a shift in reactivity would very likely be accompanied by a stiffening of the egg surface.

On the other hand, a rapid and transient rise

in the stiffness of the cell membrane at the early phase of the cleavage was reported by Mitchison and Swann (8), Hiramoto (5), and Yoneda and Dan (15) in sea urchin eggs, and by Selman and Waddington (11) in eggs of the newt, *Triturus alpestris*. Using a single pipette, the latter investigators could find no variation in the rigidity at different points on the egg surface at any stage of the cleavage cycle. Therefore, in the light of the authors' experiments, it would be interesting to check whether such stiffening occurs uniformly all over the surface, or with a time lag in different regions.

The following are the results of experiments in which stiffness was measured simultaneously at two locations in the same eggs.

## MATERIALS AND METHODS

The principle of the cell elastimeter of Mitchison and Swann (7) involved sucking out a bulge from the

egg surface with negative pressure through a small pipette. By plotting values of deformation of the surface against corresponding values of negative pressure, a straight line was obtained. The slope of the pressure-deformation curve was termed the "stiffness" which was directly related to the magnitude of the resistance to the deformation of egg surface. In order to adapt this method for the authors' purpose, a pair of pipettes was needed to measure the stiffness at two points simultaneously on the same egg. The central portion of a glass capillary was cut into halves so as to give a pair of pipettes with exactly the same inner diameter (210  $\mu\text{m}$ ). Each pipette with the sharp-cut end was mounted on a micro-manipulator and two rubber tubes exactly equal in length connected the two pipettes to a common tube to assure complete symmetry between the pair of the pipettes. This common tube was connected to a reservoir provided with a syringe (Fig. 1).

Eggs of the newt, *Cynops (Triturus) pyrrhogaster*, were used as material. Experiments were performed at room temperature (20–25°C). After the jelly capsule and the vitelline membrane had been removed, the egg was allowed to rest in Holtfreter's saline in a glass vessel fixed upon the stage of a horizontal microscope. The pipettes were brought into the glass vessel from above nearly vertically, and the levels of the Holtfreter's saline in the vessel and that of the reservoir were brought to a complete equilibrium through the orifices of the pipettes. The equilibrium was ascertained by the absence of the liquid flow within the pipettes. The pipettes were gently pressed against the top surface of the egg. The hydrostatic pressure within the pipettes was then reduced slightly

by withdrawing a small known volume of the saline from the reservoir by the syringe. In order to make complete seal between the tips of the pipettes and the egg surface, it was necessary to adjust the direction of the pipettes. Since the free surface of the saline in the reservoir was 3,030  $\text{mm}^2$  and the cross section of the syringe was 141  $\text{mm}^2$ , the movement of the piston of the syringe by 1 cm corresponded to negative pressure of 0.47 mm  $\text{H}_2\text{O}$ , or 46.1  $\text{dyne/cm}^2$ . Two bulges of the same height should theoretically be formed by the same amount of negative pressure on an inert egg, which was shown to be true (Fig. 2). Consequently, any difference in the deformation between the two points would directly indicate local difference in the stiffness.

A technical difficulty encountered was the viscoelastic property of the egg surface as pointed out by Selman and Waddington (11). On applying the negative pressure, the bulge slowly grew higher during the several minutes before the equilibrium was reached. But, since the aim of the present study was to closely follow the time-course of changes in the stiffness, the experimental procedure was modified as follows. Negative pressure was applied in four steps a minute apart, starting with  $-23.1$   $\text{dyne/cm}^2$  up to  $-50.7$   $\text{dyne/cm}^2$ , and the corresponding deformation was recorded at the end of each step by taking photomicrographs. As is shown in Fig. 3, when values of the pressure were plotted against the deformation at the end of each minute, a straight line was obtained, the slope of which was used as the stiffness (measured in  $\text{dyne/cm}^2/\text{mm}$  deformation). After four determinations were made, the suction was removed and the

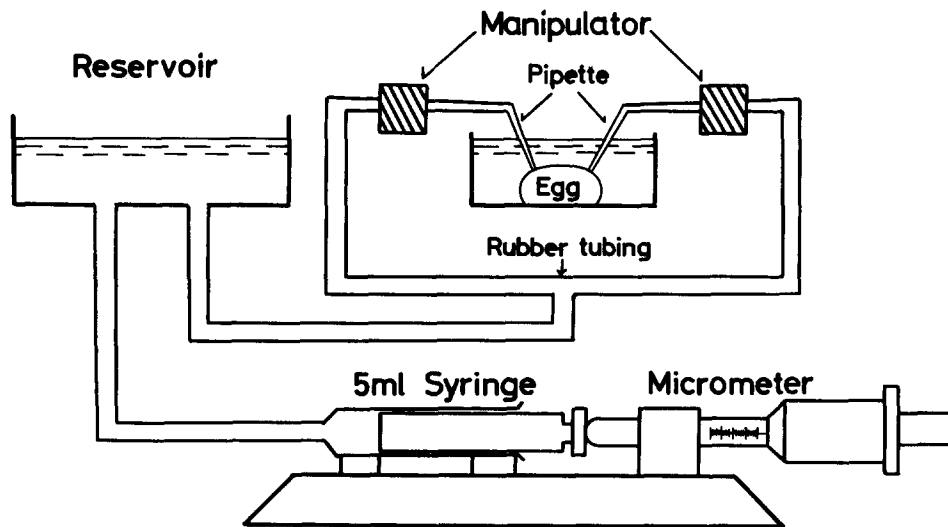


FIGURE 1 Apparatus used to measure changes in stiffness of the egg cortex at two regions simultaneously.

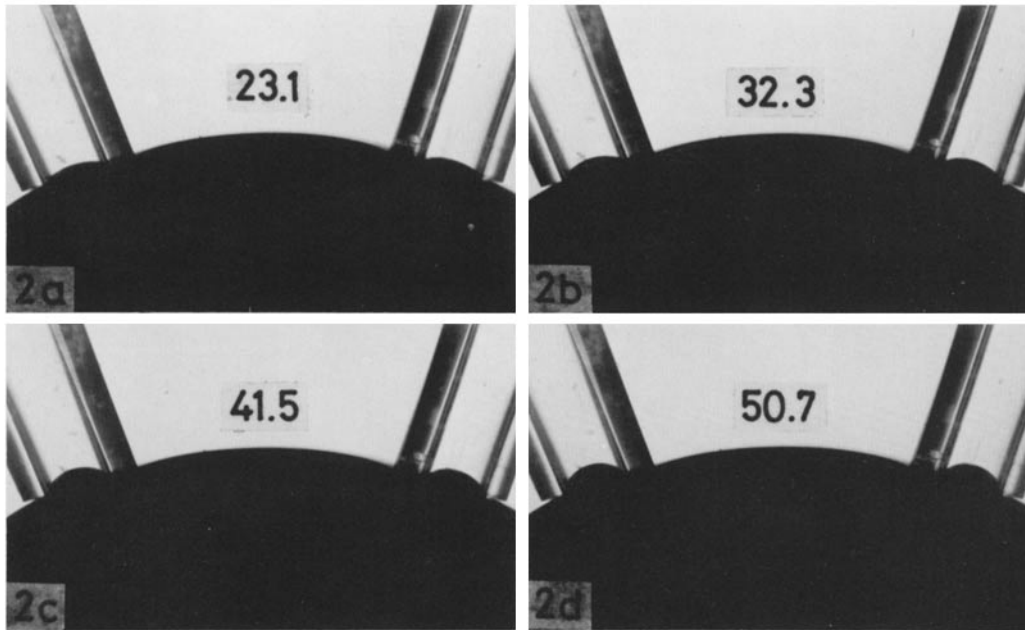


FIGURE 2 *a-d* Two bulges of the same height formed by negative pressure applied in four steps on an unfertilized egg. Numerals indicate the negative pressure in dyne/cm<sup>2</sup>. About  $\times 44$ .

pipettes were withdrawn to allow the egg surface to resume its smooth contour.

The time needed for the surface to become smooth again was usually 3–4 min, which allowed us to repeat the determination of the stiffness every 10 min on the same point. Fig. 2 also serves as a test run of the above procedure. When the cleavage furrow was travelling along the vegetal surface, however, the time for the smoothening was exceptionally long at the vegetal surface. In this case, unavoidably, points of measurement were slightly moved in each time. Selman and Waddington (11) have attributed such a long relaxation time to high viscosity of the cytoplasm at the vegetal region.

## RESULTS

Fig. 4 shows two sets of results for the changes in stiffness measured simultaneously at two points on the animal hemisphere, one being placed closer to (filled circles) and the other more distant from the animal pole (open circles). Before the cleavage, the values for stiffness in both regions were quite close to each other and changed very little. About 20 min before the start of the first cleavage, the stiffness began to rise at the point closer to the animal pole, while no change was found at the more distant point (Fig. 5 *a, b*). After about 30 min, the stiffness at the former point reached a

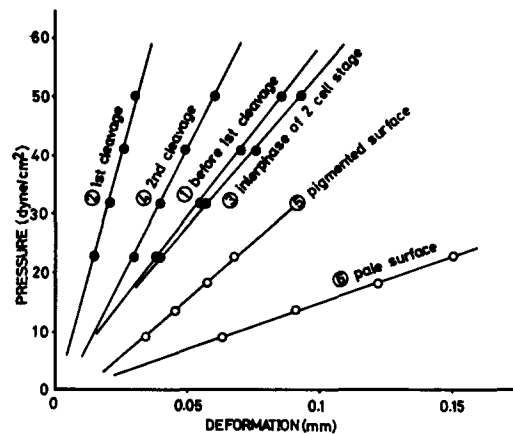


FIGURE 3 Pressure-deformation plottings in *Cynops* eggs. (1)–(4): Measurements on the animal half at four stages of the same egg. (5)–(6): Simultaneous measurements with another egg on the pigmented and unpigmented surfaces of the furrow wall during the first cleavage. Ordinate, negative pressure (dyne/cm<sup>2</sup>). Abscissa, deformation of surface (mm).

maximum value 2–3-fold of the original one, and at the latter point the beginning of the increase in the stiffness could be perceived (Fig. 5 *c*). In a short time, the stiffness at the former point fell

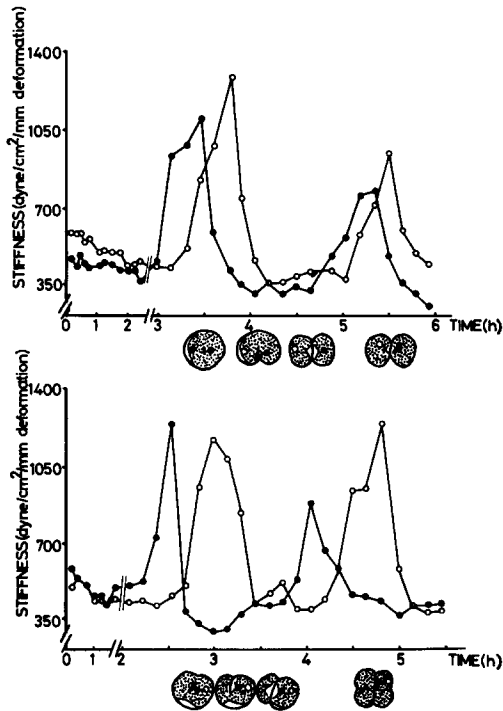


FIGURE 4 Changes in stiffness of the cortex at two points of different latitude on the animal half, during the first two cleavage cycles. Ordinate, stiffness (dyne/cm<sup>2</sup>/mm deformation) shown by the slope of the pressure-deformation curve. Abscissa, time (h) (time 0 is the start of measurement, and the scale during the quiescent period is shortened). Figures at the bottom show the progress of the furrow and the two sites of measurement.

sharply roughly to the level before the first cleavage, when the stiffness at the latter point reached a maximum (Fig. 5 *d-f*). In other words, the stiffness at the two points varied similarly, but with a time lag of about 30 min. For the second cleavage, a similar time lag in the change in the stiffness was repeated.

Results obtained from measurements at two points, one on the animal and the other on the vegetal region, are given in Fig. 6. Here again the rigidity rose earlier at the point nearer to the animal pole.

In the measurements on two points of the vegetal hemisphere, the cortex nearer to the equator became rigid earlier than the lower region (Fig. 7). However, it was noted that the vegetal surface showed slight unevenness while the wave of high stiffness was passing, after which the surface smoothed itself again (Fig. 8). Such an unevenness of the surface may cause a slight

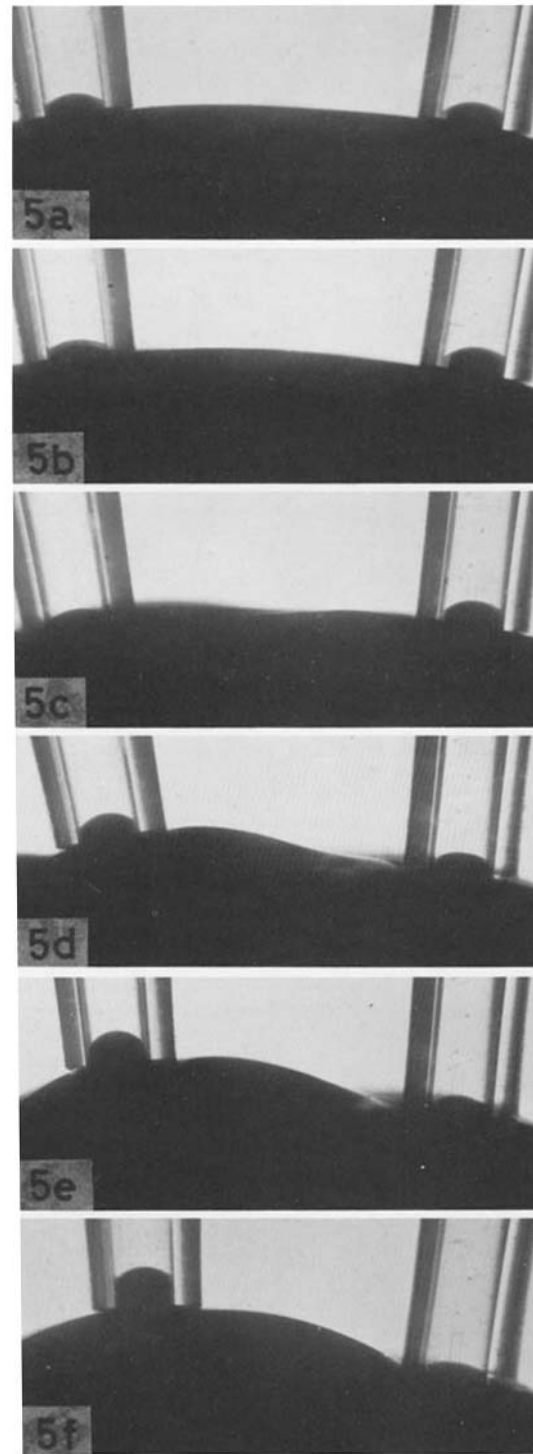


FIGURE 5 Changes in stiffness at two points, one being closer to (left) and the other more distant from the animal pole (right). Interval between successive photographs: 10 min. Pressure: 50.7 dyne/cm<sup>2</sup>. Stiffness

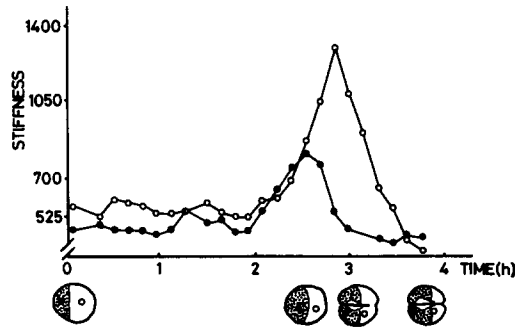
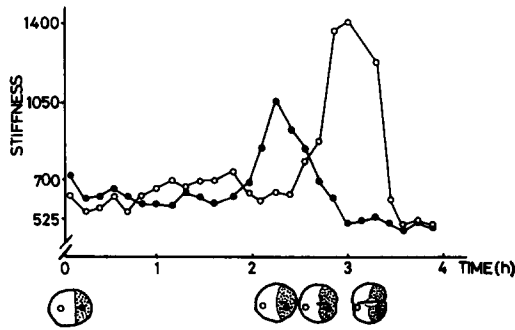


FIGURE 6 Changes in stiffness at two points, one on the animal and the other on the vegetal half during the first cleavage cycle. Ordinate and abscissa, the same as in Fig. 4.

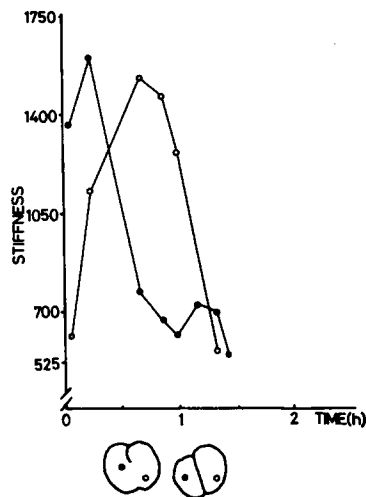


FIGURE 7 Changes in stiffness at two points in different latitude on the vegetal half during the first cleavage. Ordinate and abscissa, the same as in Fig. 4.

(dyne/cm<sup>2</sup>/mm deformation): (a) left, 525 and right, 434, (b) 721 and 413, (c) 1,246 and 455, (d) 385 and 504, (e) 329 and 952, (f) 294 and 1,169.

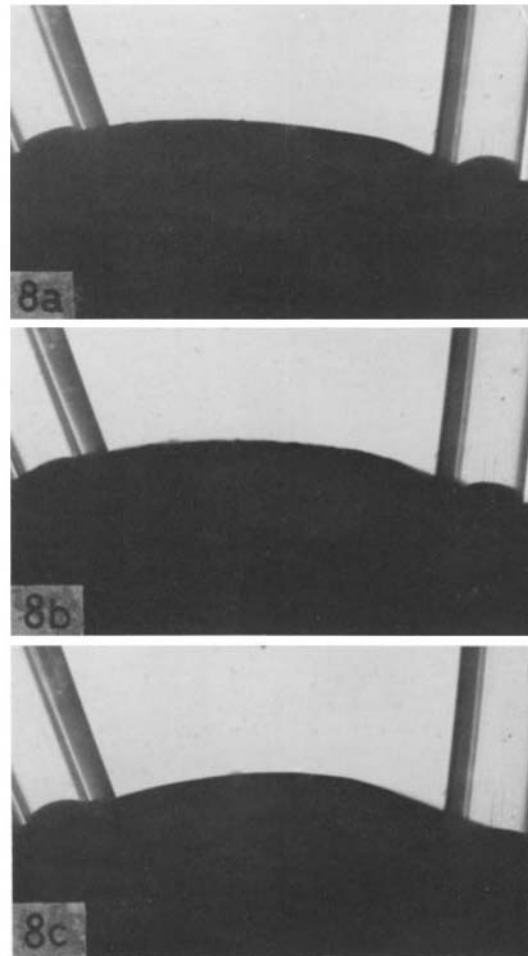


FIGURE 8 Changes in stiffness and contour at the vegetal pole region. (a) stiffness begins to rise at left point and surface becomes slightly rough on the left side, but no change on the right side. (b) 14 min after (a), stiffness reaches a maximum at the left and it begins to rise at the right. Rough contour over the entire vegetal surface. (c) 10 min after (b), at the right, stiffness reaches a maximum and the surface becomes smooth again. Pressure: 50.7 dyne/cm<sup>2</sup>. Stiffness (dyne/cm<sup>2</sup>/mm deformation): (a) left, 1,295 and right, 609, (b) 1,610 and 1,113, (c) 777 and 1,540.

leakage between the pipette and the surface which possibly results in the overestimation of the measured stiffness. In Fig. 6, the peak value for the stiffness of the vegetal surface was higher than the peak for the animal surface. The high stiffness may be caused by high viscosity of cytoplasm at the vegetal region as suggested by Selman and Waddington (11). However, further confir-

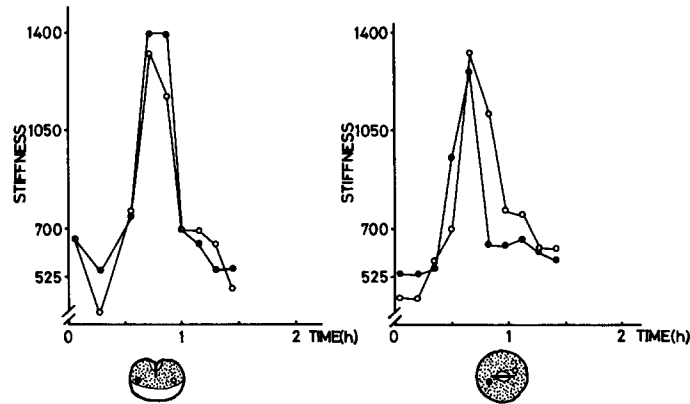


FIGURE 9 Changes in stiffness at two points symmetric against the furrow tip on the same latitude. Ordinate and abscissa, as before.

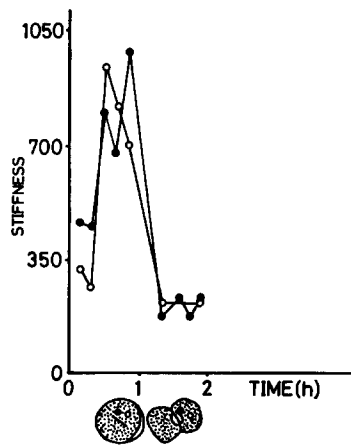


FIGURE 10 Changes in stiffness at two points on the same latitude but asymmetric against the furrow tip. Ordinate and abscissa, as before.

mation of this point would be required for the above-mentioned reason.

In contrast to the preceding experiments, if a pair of the pipettes was applied on the same latitude, coincidence of the time feature of the change in the stiffness at the two points was evident (Figs. 9, 10). In Fig. 9, two points were symmetric against the furrow tip on the same latitude, while in Fig. 10, two points were on the same latitude but asymmetric against the furrow tip.

From the above results, it was concluded that there was a wave of high stiffness which propagated as a belt, starting from the animal pole toward the vegetal pole. Roughly speaking, the advancing tip of the cleavage furrow was on the same latitude as the belt of high stiffness as schematically shown in Fig. 11.

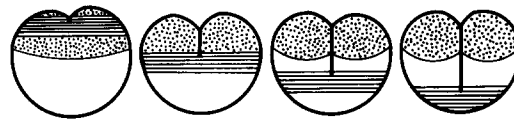


FIGURE 11 Diagram showing travel of a belt of high stiffness (horizontal bars) during the first cleavage.

Before going further, some comments will be made on the pale surface appearing on both sides of the deepening furrow. The pale surface was found to be softer than the other surface (Fig. 3) in all the simultaneous measurements so far made. Continuous measurement at the pale surface was difficult, however, since the surface adhered to the tip of the pipette. Such a stickiness and a low stiffness have been found by Selman and Waddington (11) at the region of "the forming furrow" in areas of new unpigmented surface. Difference in ultrastructure between pigmented and new unpigmented surface has been reported by Selman and Perry (10), that is, extracellular material adhered abundantly to the pigmented surface and less to the new surface.

## DISCUSSION

The present results revealed the presence of the wave of the high stiffness which travels, as well as the wave of furrow-forming reactivity (9), down meridionally as a belt from the animal pole toward the vegetal pole in accompaniment with progress of the cleavage furrow. Rough coincidence in time of appearance and propagation of both waves seem to indicate that the increase in the stiffness is an expression of some preparation neces-

sary for the cleavage furrow formation. By time-lapse cinematography, Hara (4) found, in axolotl egg, a narrow circular zone of concentrated pigment which propagates as a wave from the animal pole toward the vegetal pole with advance of the cleavage furrow. In the newt eggs, the present authors have confirmed a similar wave (unpublished).

These various waves of change start from the animal pole region, below which the mitotic apparatus is situated, and it is highly probable that some stimulus causing these changes is released from the mitotic apparatus before cleavage and continues to spread along the cortex, once it is started. Probably, the capacity for forming the cleavage furrow is distributed basically within the area of high stiffness but realization of the furrow is specifically limited to where the "furrow inducing cytoplasm" (9) is located within the area. While several workers (1-3, 6, 10, 12-14) have demonstrated bundles of microfilaments at the bottom of cleavage furrow in various type of eggs, Bluemink (2) further observed such filaments at the surface which is not involved in furrow formation, though not in bundles. This may also indicate that the capacity for forming furrows is potentially distributed over the cortical area including the filaments.

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