A STUDY ON THE MECHANISM OF CLEAVAGE IN THE AMPHIBIAN EGG*

BY K. DAN† AND M. KUNO KOJIMA‡

In comparison with the large number of investigations performed on the cleavage of sea-urchin eggs, those on the cleavage of amphibian eggs are surprisingly scanty. Recent works are almost limited to two papers from Edinburgh by Waddington (1952) and by Selman & Waddington (1955). The present report is an amplification of the works of the British investigators.

MATERIAL AND METHOD

The analysis was focused on the early cleavages of the egg of the newt, *Triturus pyrrhogaster*. After removing the jelly and the vitelline membrane, the eggs were cultured in Holtfreter's solution in a dish, the bottom of which was covered with agar gel. When necessary, they were operated on in the same solution.

RESULTS

I. *Confirmation of the cellophane experiment of Waddington*

For the purpose of testing whether the impetus for furrow formation is given from the cell interior to the cortex or arises within the cortex independent of the interior, Waddington inserted a piece of cellophane into the egg in a tangential direction, causing the cellophane strip to pierce the egg at a relatively shallow level and effect a separation of the cortex from the endoplasm. Under these conditions the cleavage furrow was found to pass over the cellophane strip, suggesting that its progress is independent of the cell interior.

The present authors repeated this experiment and fully confirmed Waddington's conclusion. However, since a few subsidiary phenomena came to our notice, a brief description of them will be given.

(a) *Extrusion of the cellophane strip*

In order to minimize the influence of operational shock on the cleavage activity, the cellophane strip was inserted well ahead of the tip of the advancing furrow, to allow ample time between its insertion and the onset of cleavage. When this was done, however, the cellophane strip was found to be ejected by the egg, in such a way that the belt of cortical cytoplasm going over the cellophane was gradually reduced in width until it finally became a narrow band of cortical material surrounding it. At this point, the cellophane strip usually lost its balance and tilted to one side or fell completely away from the egg surface, leaving a small black cytoplasmic protrusion. Waddington

described this by saying, 'It may be that the cut edges of the coat which lie underneath the cellophane tend to flow inwards below it, thus slowly pushing it out of the egg' (p. 487). At any rate, when a cellophane strip comes out spontaneously, no open wound is left where it was last connected to the egg.

A point of interest here is the fact that this ejection proceeds much faster on the animal than on the vegetal area in the eggs of *Triturus pyrrhogaster*. But whether this is due to a stronger contractility of the animal cortex or is the result of the higher level of general metabolism of this region cannot be said at present.

(b) Complete bisection of the cortical layer at the furrow site

When transparent cellophane is used, all one can detect is that the furrow passes over the cellophane. However, when coloured cellophane is used, particularly on the pale vegetal region, the result becomes more spectacular. In our experiment, red cellophane was used. As long as the cellophane strip was buried under the cortex, the colour was not visible, but once the furrow had passed over it, the colour was revealed brilliantly, indicating that the cortical layer had been clearly separated into two parts. This is illustrated in series A of Pl. 1. It may be added that the results are the same, whether the wounds heal perfectly as shown here, or remain unhealed.

II. *Effect of incision across the path of the furrow*

Waddington studied the effect on divison of cutting the cortex in two ways: viz. either parallel or transverse to the cleavage furrow. The results obtained in the latter case are very suggestive, but Waddington's description of them is unfortunately rather brief. He says: 'In another small series of eggs cuts were made perpendicular to the furrow, as in Fig. 3. Some 10 min. later, while the cuts were still gaping open, the lateral parts of the furrow were visible outside the cut regions' (p. 486).

With the idea of examining this case more closely, the present authors varied the position of the incision with reference to the tip of the advancing furrow. When a cut is made across the future path of the furrow fairly far beyond its tip, the furrow stops on reaching the edge of the cut. If, however, the cut is made either across the already-formed furrow or only a short distance ahead of the furrow tip, the furrow jumps across the gaping wound, so to speak, and appears on the other side of it to continue its previous course (series B). The last example of Waddington's experiment (iii), in which the furrow continued to progress after removal of its early-formed portion, must be included in this category. Experiments made to determine the critical distance from the head of the furrow, beyond which the furrow will be stopped by the wound, gave a value of about 1 mm. (see Table 1).

The above results can be interpreted as showing that within the range of 1 mm. from the furrow tip, some sort of preparation for furrow formation must be going on. As the result, when this area extends to the far side of the cut, black pigment begins to accumulate and a furrow can be initiated from there. The furrow is presumably stopped by a cut made more than 1 mm. away from its tip because the organizing influence of the furrow tip cannot be transmitted across a wound, where the cortical continuity has been broken.

However, even in this case, the unimpeded furrow tip at the other side of the egg

continues to advance and, after passing over the vegetal pole, eventually reaches the opposite side of the wound (series C). Although the end result looks identical in series B and C, there is no danger of misjudging whether the original furrow has succeeded in crossing the cut or whether the other end has come around from the vegetal side, since the pigment is always pulled toward the tip of an approaching furrow (compare series B and C; see also Selman & Waddington, 1955).

A rather surprising thing is that although the unoperated part of the furrow has to travel more than half the circumference of the egg, and cross the vegetal pole, where it ordinarily stops, it always arrives at a point straight across the wound from the point where the other furrow is stopped. This shows that the furrow is somehow endowed with the capacity to navigate along a straight course.

One of the common denominators of the finding described in sections I and II can be summarized as follows: the furrow continues along a straight course, as long as the material continuity of the cortex is preserved.

Table 1. *Positions of incisions ahead of furrow tip and the behaviour of the furrow*

	Behaviour of the furrow		
Distance by micrometer scale	[Successful passage across the cut	Tendency to go around the cut	Complete stoppage by the cut
5	5	—	—
5–8	1	—	—
8	1	1	—
8–10	1	—	—
10	—	—	6

1 micrometer scale unit corresponds to 0·11 mm.

III. *Passing over rows of pricks*

Instead of making an incision, we pricked the egg surface with a fine glass needle in rows across the future path of the furrow. Two or three rows were made more than 1 mm. away from the furrow tip, and the positions of the pricks in one row were so chosen that they would alternate with the pricks of the adjacent rows. In spite of these obstacles, the furrow was found to cross the pricked zone without any hindrance whatsoever.

Under a binocular dissecting microscope the wounds are easily recognizable because granular yolk spheres are visible within each pit. When the furrow tip approaches the pricked zone, the nearby pits suddenly vanish or, more strictly speaking, the granular yolk is suddenly covered by a smooth shiny surface like that of the rest of the egg. In other words, as the furrow advances the pits in its path are filled, although the more distant ones remain unchanged. Oblique rows of prickings were made in an attempt to make the furrow deviate from the straight course, but this was never successful.

This experiment shows that the advance of a furrow along its straight course must be understood as a very positive process, of laying down a straight track ahead of the furrow, and not as a casual use of any tracks which happen to become available.

IV. *Passing under a weight*

As a check on the above conclusion, a piece of rather thick glass rod was laid across the egg to act as a weight, in order to see whether the propagation of the furrow is possible under pressure when the structural continuity of the cortex is maintained intact. That this is indeed the case is illustrated in series D of Pl. 2.

V. *Successful passage of the furrow over a narrow bridge of cortex*

To test this idea two large cuts were made on the two sides of the median plane of the egg, leaving a narrow strip of intact cortex in the middle strictly on line with the furrow. This experiment showed that, however narrow a strip might be, the furrow was able to advance as long as intact cortex was provided for its passage.

Naturally, approaching furrows often missed the approach to the bridge and stopped when they arrived at the side of the wound. Such failures were mainly due to geometrical inaccuracy in making the bridge exactly on the line of approach of the furrow. A more important feature of this experiment, however, is the observation that when geometrical accuracy was achieved the furrows crossed such narrow bridges and with a far higher frequency than was anticipated.

After the furrow has traversed the narrow black strip of cortex, this is further split lengthwise by the appearance of the secondary furrow in Schechtman's sense; this means that the cleavage furrow is perfectly normal in every respect. At present, series E is the only available photographic record of this process; although it is not the most impressive case, it shows all the essential features.

Waddington found that the furrow was not disturbed by two parallel cuts made on either side of it (his experiment (ii)). The present result indicates that a furrow can even go through a narrow space between two cuts set some distance ahead of it.

VI. *Passing over an oblique bridge*

When a narrow bridge of cortex is formed in such a way that only its proximal end lies in the presumptive furrow plane while the rest of it runs obliquely to the furrow, two kinds of results are obtained.

(a) *When the wound remains unhealed*

Since the direction of the bridge is so selected, the furrow tip usually reaches its proximal end. But as it follows its own straight course over the bridge, it sooner or later falls to the side. If the incision into which the furrow falls has not healed, the furrow is prevented from further advance. The outcome is the same as that described in section II.

(b) *When the wound is healed*

Although detailed observation of the healing process has so far proved impossible, it can be seen with a dissecting microscope that the granular appearance of the exposed yolk is lost, as though an invisible membrane is spreading over the yolk granules from the edge of the wound close to the furrow tip. When this happens the

furrow is able to pursue its pre-determined course, even though this departs from the bridge.

A very strange feature of the healing phenomenon under these conditions is that very often the covering of the wound occurs only to a sufficient extent to permit the furrow to advance straight, so that it runs along the border between the healed and unhealed parts (consult series F). Those who are familiar with amphibian eggs may have noticed that when an egg with a median injury cleaves, the wound extends all the way to the furrow in one of the daughter blastomeres, whereas the opposite blastomere is completely free from injury. This sort of thing may well be a direct consequence of a recovery process of the kind described here.

VII. *Advance of the furrow on excised pieces of cortex*

The notion arrived at so far suggests that furrowing ought to be initiated even in an excised piece of cortex, if a furrow tip or a portion bordering it is included in the piece.

This expectation was borne out in practically all the cases tried as shown in series G and H of Pl. 3. This technique should be very useful in future for the study of the cleavage process, since it is possible to observe the furrow in cross-section as well as from the underside, which is utterly impossible to do in an opaque intact egg. A further analysis of this case is being planned.

VIII. *Transplantation of non-equatorial cortex to the equatorial region of the egg*

A transplantation experiment was done to test the possibility that the furrowing activity may pass into non-equatorial cortex. Since preliminary attempts to follow Curtis's method (1960) were not successful, a piece of non-equatorial cortex with some yolk granules was placed on the equatorial region of another egg, the cortex of which had been removed. After some practice this technique could be used with sufficient skill so that the contact line between the original and transplanted pieces of cortex can be made neatly with neither excess nor deficiency of the cortical material, at least on the side facing the oncoming furrow. In spite of this, no case has so far been obtained in which a furrow was formed in the transplanted cortex. However, when a furrow abuts against the edge of the transplant, there is always some movement of the pigment on the transplanted piece somewhat simulating that occurring prior to normal furrowing. The results, therefore, cannot be disposed of as entirely negative, and require further study.

DISCUSSION

It has been shown in this paper that: (1) the advancing tip of the cleavage furrow of the *Triturus pyrrhogaster* egg is preceded by an area less than 1 mm. in length where some kind of preparation for furrowing is going on; (2) the advance of the furrow is strictly along a straight course; (3) the structural continuity of the cortex is a requisite for the propagation of the furrow.

Within the extent of the above findings it is permissible to conclude, as Waddington did, that the furrow propagates from the animal hemisphere toward the vegetal via the cortex alone, and no intervention of the endoplasm is necessary. However, when one tries to explain the first appearance of the furrow at the centre of the animal hemisphere,

this statement is not self-evident. To explain the initiation of the furrow, it may be necessary to invoke some agency other than the cortex, such as the nucleus.

In the newt egg, this possibility is suggested by the fact that when the egg is deprived of its vitelline membrane rather early, leaving it in a flattened condition for a long time before it begins to cleave, the cleavage furrow appears at an abnormal position, quite often near the periphery of the pigmented part. Presumably this is due to the abnormal position taken by the synkaryon because of the extreme flattening of the egg.

However, although the influence from the nucleus, if there is any, would easily permeate the entire cell body as long as the cell is small, this would not necessarily be the case in a large egg of the discoidal type of cleavage, necessitating the adoption of an auxiliary method such as the self-propagating capacity of the furrow reported in this paper.

In the field of sea-urchin cleavage, on the other hand, the situation seems to be as follows: shortly before the appearance of the cleavage furrow, a local differentiation can be perceived in the cortex (Kuno, 1954a; Kuno-Kojima, 1957). Once such a cortical differentiation is established, the cell is capable of cleaving in the absence of a mitotic apparatus (Beams & Evans, 1940; Swann & Mitchison, 1953; Kuno, 1954b; Hiramoto, 1956). A detergent like Monogen can cause dedifferentiation, with the consequence of suppressing the cleavage (Kuno, 1954b; Kuno-Kojima, 1957). The cause which gives rise to such a cortical differentiation cannot simply be ascribed to a nuclear factor, since the same differentiation takes place in enucleated fragments activated by parthenogenetic agents (Kuno-Kojima, 1957).

At any rate, by considering the message from the nucleus (and/or the endoplasm) as the primary cause for cleavage, which works regardless of the size of the egg, and the self-propagating capacity of the amphibian cleavage furrow as a secondary adaptation for large cells, it is possible to put the division of sea-urchin eggs and that of amphibian eggs on common ground. However, if this view is accepted a third point must also be added as a corollary for amphibian ova; viz. the advance of the furrow along the cortex must be followed by the centripetal growth of a septum to achieve a complete separation of the blastomeres. The diasteme demonstrated by Selman & Waddington (1955) in the newt egg may represent this phase, although their account is, so far, a purely morphological one.

One other aspect of amphibian cleavage brought to light by this study which has no counterpart in the cleavage of sea-urchin eggs may need some emphasis. This is the clear-cut bisection of the cortex by the furrow that was found in two of the experiments described above, in the appearance of the red colour of the cellophane strip through the cleavage furrow (series A) and the lengthwise splitting of the narrow bridge of pigmented cortex after a furrow has traversed it (series E). Although the cellophane experiments have not been tested on isolated cortex, the division of excised pieces of the cortex is no other than a further substantiation of the experiments of the series E type. Such separation must then be considered to be really autonomous and an intrinsic capacity of the furrow cortex, since the possiblity that some outside force is tearing the cortex is completely eliminated in the excised pieces. In other words, this is a spontaneous transformation of 'oneness' into 'twoness'.

Many theories have been proposed to explain the mechanism of the cleavage of sea-

urchin eggs (Wolpert, 1960). Whether the force is postulated as originating from the equatorial ring or as coming from other parts of the egg, the concept of the division process which such theories embody is, after all, a forcible pulling apart of the cell contents into two portions and none of these theories considers the possibility of an autonomous separation of the cell structure, except in the cases of the centrioles (Mazia, Harris & Bibring, 1960) and the chromosomes. But even there, the separation of these units and the chromosomes is considered as an indirect preparation for the beginning of cleavage rather than as an immediate cause of it. This blind spot in the theoretical approach may well explain why theories put forward to explain the mechanism of division in the sea-urchin egg have failed to arouse much interest among the students of amphibian eggs in the past.

SUMMARY

1. In the eggs of the salamander, *Triturus pyrrhogaster*, there is an area extending about 1 mm. ahead of the advancing tip of the cleavage furrow where preparation for furrow formation is going on.

2. Severance of this area ahead of an advancing furrow tip does not stop the progress of the furrow. The furrow continues from this area.

3. The cleavage furrow of *Triturus pyrrhogaster* eggs advances strictly along a straight line.

4. A cut made across its path ahead of a furrow is healed as the furrow tip approaches sufficiently to allow its straight passage.

5. An excised piece of cortex which includes material taken from within 1 mm. of an advancing furrow tip will form a furrow and divide.

REFERENCES

BEAMS, H. W. & EVANS, T. C. (1940). Some effects of colchicine upon the first cleavage in *Arbacia punctulata*. *Biol. Bull., Woods Hole*, **79**, 188–98.

CURTIS, A. S. G. (1960). Cortical grafting in *Xenopus laevis*. *J. Embryol. Exp. Morph.* **8**, 163–73.

HIRAMOTO, Y. (1956). Cell division without mitotic apparatus in sea-urchin eggs. *Exp. Cell Res.* **11**, 630–6.

KUNO, M. (1954*a*). On the nature of the egg surface during cleavage of the sea-urchin egg. *Embryologia*, **2**, 33–41.

KUNO, M. (1954*b*). Comparative studies on experimental formation of multinucleated eggs of sea urchins by means of various agents. *Embryologia*, **2**, 43–9.

KUNO-KOJIMA, M. (1957). On the regional difference in the nature of the cortex of the sea-urchin egg during cleavage. *Embryologia*, **3**, 279–93.

MAZIA, D., HARRIS, P. J. & BIBRING, T. (1960). The multiplicity of the mitotic centers and the time-course of their duplication and separation. *J. Biophys. Biochem. Cytol.* **7**, 1–20.

SELMAN, G. G. & WADDINGTON, C. H. (1955). The mechanism of cell division in the cleavage of the newt's egg. *J. Exp. Biol.* **32**, 700–33.

SWANN, M. M. & MITCHISON, J. M. (1953). Cleavage of sea-urchin eggs in colchicine. *J. Exp. Biol.* **30**, 506–14.

WADDINGTON, C. H. (1952). Preliminary observations on the mechanism of cleavage in the amphibian egg. *J. Exp. Biol.* **29**, 484–9.

WOLPERT, L. (1960). The mechanics and mechanism of cleavage. *Int. Rev. Cytol.* **10**, 163–216.

EXPLANATIONS OF PLATES
Plate 1

Series A. Successful passage of a furrow over a piece of red cellophane. The furrow had not yet reached the region overlying the cellophane strip when the operation was finished. Temp. $16.5°$ C. Time A_1, o min.; A_2, 14 min.; A_3, 28 min.; A_4, 56 min.

Series B. Insertion of a cellophane strip (partly red and partly white) perpendicularly to the egg surface very close to the tip of a furrow. Note the initiation of the furrow on the opposite side of the gaping wound preceded and accompanied by accumulation of pigment. Note also that the egg is flattened as a result of the removal of the vitelline membrane. Temp. $15.5°$ C. Time: B_1, o min.; B_2, 11 min.; B_3, 20 min.; B_4, 29 min.

Series C. Prevention of the advance of a furrow by an incision made farther than 1 mm. away from the furrow tip, and the arrival of the opposite tip of the furrow from underneath. Temp. $15.5°$ C. Time: C_1, o min.; C_2, 9 min.; C_3, 22 min.; C_4, 51 min. Compare the positions of the cuts in reference to the furrow tips between B_1 and C_1 and also the times of B_4 and C_4.

Plate 2

Series D. Continuation of furrow formation under a weight. Temp. $15.5°$ C. Time: D_1, o min.; D_2, 16 min.; D_3, 27 min.; D_4, 54 min.

Series E. Passage of a furrow through a narrow strip of intact cortext left between two large incisions. In E_3, the bridge of pigmented cortex is split lengthwise after passage of the furrow. Temp. $16.0°$ C. Time: E_1, o min.; E_2, ? min.; E_3, 17 min.

Series F. Passage of a cleavage furrow with a partial healing of wounds. Temp. $14.5°$ C. Time: F_1, o min.; F_2, 18 min. The furrow misses the cortical bridge on which the pigment is accumulating. F_3, 25 min. On the upper right corner of the left wound, healing has taken place; this, however, could not be shown in the photograph. F_4, 61 min. Note that the left side wall of the furrow is in direct contact with the left wound, with no intervening pigmented strip; this indicates that the furrow advanced on the border line between the healed and unhealed parts during the stage shown in F_3.

Plate 3

Series G. Piece of cortex removed from area ahead of furrow which is cleaving larger fragment. Note that the piece cleaves. Temp. $14.5°$ C. Time: G_1, o min.; G_2, 10 min.; G_3, 33 min.; G4, 76 min.

Series H. The same as series G. In G_1 and H_1, no trace of the furrow could be detected in the isolated portion immediately after the operation. Temp. $11.0°$ C. Time: H_1, o min.; H_2, 12 min.; H_3, 50 min.; H_4, ? min.

K. DAN AND M. KUNO KOJIMA

K. DAN AND M. KUNO KOJIMA