# PRELIMINARY OBSERVATIONS ON THE MECHANISM OF CLEAVAGE IN THE AMPHIBIAN EGG

By C. H. WADDINGTON
*Institute of Animal Genetics, Edinburgh*

(*Received* 5 *December* 1951)

(With Five Text-figures)

The formation of blastomeres by the newly fertilized egg has been studied by many authors, who have found the process a convenient avenue for attacking the general problem of cell division. By far the greater part of the work has been carried out on the eggs of marine invertebrates, and, in spite of their great suitability for most types of experimental embryological investigation, the eggs of Amphibia have not as yet been made much use of in this connexion. The nuclear phenomena attendant on fertilization and cleavage in the Amphibia have been rather fully described (see, for instance, Fankhauser & Moore, 1941), but a short note by Schechtman (1937) contains almost everything that we know about the cleavage of the cytoplasm, and the part played in it by cortical movements.

During the last two summers a few experiments have been made which show that the amphibian egg provides very favourable material for a direct attack on certain of the crucial problems of cell division, since not only is it rather easy to perform micro-surgical operations on the developing cleavage furrow in a manner which would be very difficult in the small eggs of most invertebrates, but the pigmentation of the surface allows the cortical movements to be followed precisely in time-lapse cinema films. These experimental opportunities have not yet been by any means fully exploited, but the preliminary experiments have already yielded certain results which it seems worth while to record.

The experiments were made with eggs of *Triturus alpestris* which were laid and fertilized normally in the aquarium. The eggs were observed in Holtfreter solution, either of normal strength, or diluted with an equal volume of distilled water.

## OBSERVATIONS

The time of fertilization of the eggs being not exactly known, the onset of cleavage could not be foretold in advance. The first sign of it which is visible externally is the appearance of a thin dark line in the neighbourhood of the animal pole. This marks the beginning of the cleavage furrow and, as is made clear by cinema films, it is formed by a gentle movement of the cortex from each side towards the line. It constitutes the 'primary furrow' of Schechtman, and as he notes, it is soon replaced by a 'secondary furrow', in the formation of which the cortex condensed in the line begins to expand, so that the line broadens, becomes paler, and begins to sink into the egg.

A series of experiments was performed on eggs in which the primary furrow had just appeared or was in process of being transformed into the secondary furrow. After removal from the jelly capsule, an egg at this stage is contained in the vitelline membrane and has the shape of a sphere considerably flattened in the animal pole region, where there is a space between the surface of the egg and membrane. If the membrane is completely removed, the egg settles down as a rather flat bun-shaped object, far removed in shape from a sphere. Cleavage may nevertheless be performed quite well, the two daughter blastomeres tending to fall apart under their own weight. In most of the experiments to be described, the vitelline membrane was removed only from the animal half of the egg or in some cases was left more or less intact, being cut through only sufficiently to allow the operation to be performed; in such circumstances what remains of the membrane helps to hold the egg into a more nearly spherical shape.

(i) An opening was made into the egg in the region more or less in line with one pole of the spindle. This is near the equator of the egg in the usual embryological sense, which takes the animal pole as its main point of reference. (There is a possible ambiguity in terms to be guarded against here, since the axis of the spindle is perpendicular to the axis of the egg.) Through this opening some of the interior cytoplasm of the egg is extruded. In several of the experimental eggs this extrusion was assisted by raking out more cytoplasm with a needle until about a quarter of the internal contents of each half of the egg had been removed. After this had been done the egg was quite flaccid, appearing in every way as though its surface was too large for the material left inside it. The wounds made during the operation did not close and heal unless they were fairly small.

The cleavage continued apparently normally until the egg was cut into two blastomeres (Fig. 1). The same result occurred when openings were made over both poles of the spindle, so that both the first two blastomeres were partially emptied. Injured blastomeres usually died before the onset of the second cleavage would be expected.

(ii) In a number of eggs in which the furrow extended over only about a fifth of the diameter, two cuts were made through the cortex parallel with the furrow, in the positions shown in Fig. 2. As Holtfreter (1943) has described, the first result of a cut through the surface coat is a retraction of the latter; this is shortly afterwards followed by a contraction of the wound, which fairly rapidly becomes completely closed. A similar series of events was noted here. In some cases the cleavage continued more or less normally, at least as far as could be seen externally, but in some eggs the cleavage furrow appeared to be shallower and to extend less deeply into the interior in the region of the wound. In all cases the closure of the wound took place before the furrow had penetrated very deeply into the egg.

(iii) In a few eggs (only three) the first sign of the cleavage furrow was completely extirpated, the parallel cuts being located rather closer to it than those shown in Fig. 2. In two cases, in which the wound healed rather rapidly, the cleavage appears to be quite normal. In the third the wound healing was delayed, and it was noted that the cleavage furrow appeared on the lateral parts of the egg

at a time when at the animal pole there was no sign of a furrow, the wound being still open.

(iv) 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.

(v) In a number of eggs a needle was inserted and waggled from side to side under the region of the primary furrow, so as to cut any material connexion there might be (by way of astral fibres, for instance) between the cortex and the more deeply lying structures. Cleavage continued quite normally.

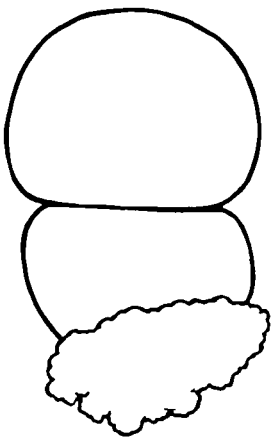

Fig. 1.

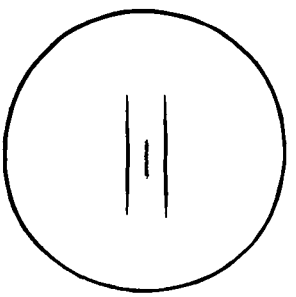

Fig. 2.

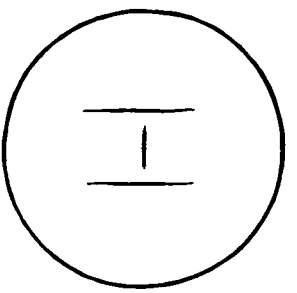

Fig. 3.

Fig. 1. Egg opened at equator (towards lower side of drawing) and some of the internal cytoplasm allowed to escape. Normal cleavage.

Fig. 2. Operation to an egg at the stage of the first sign of the cleavage furrow; cuts through the cortex parallel to the furrow.

Fig. 3. Cuts perpendicular to the furrow.

(vi) Two cuts were made, one on each side of the furrow, and located some distance away from it. By means of a needle, a path was cleared through the cytoplasm from one cut to the other, and a piece of cellophane carefully worked through so that it penetrated the egg from one side to the other. The thickness of the cellophane was approximately 0·08 mm., and the width of the strips used was about a quarter to a third of the egg diameter. The wounds through which the cellophane was passed naturally remained open, and cytoplasm extruded slowly through them. The cleavage furrow, however, continued to deepen, even though entirely separated from the interior by the strip of cellophane. Moreover, if the strip lay somewhat to one side of the furrow, the latter was observed to extend over it (see Fig. 4). So far as could be seen, the furrow extended downwards till it came in contact with the cellophane; unfortunately, it did not prove possible to fix and prepare the specimens for sectioning without so much injury that it was not possible to discover the conditions below the cellophane.

In the later development of these eggs, two phenomena were observed. After

some time the cleavage furrow seemed to disappear over the region underlain by the cellophane. But this was somewhat obscured by the fact that the cellophane itself did not remain in place without disturbance. It was, in fact, gradually worked up towards the surface of the egg and eventually extruded completely. The mechanism of this action is obscure. 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; it would appear probable, however, that some active reaction of the cell interior is also involved.

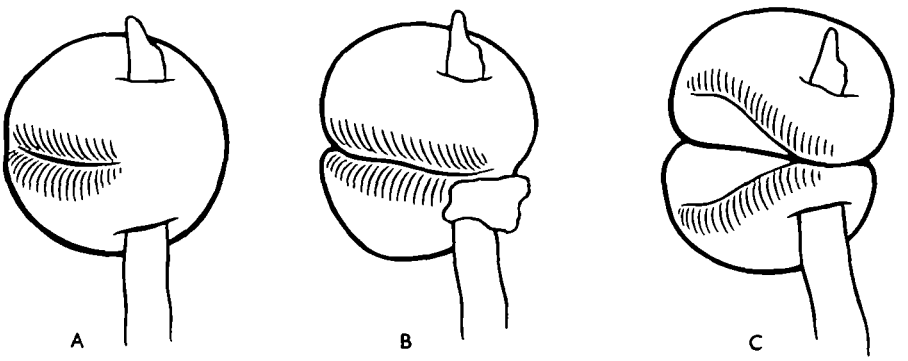


Fig. 4. A strip of cellophane inserted through an egg, not far beneath the cortex. A, at 3.23 p.m., a few minutes after the operation which was performed when the cleavage furrow was about half the length shown in the drawing; B, at 3.42 p.m.; C, at 3.50 p.m.

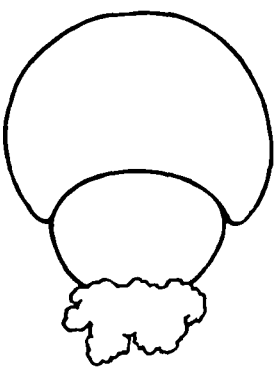

Fig. 5. Egg opened at equator (towards lower side of drawing) and an attempt made to pull the cleavage spindle towards the opening.

(vii) In another series of eggs a cut was made near the egg-equator, over what should be one of the poles of the spindle. With a needle, some cytoplasm was pulled out, and the needle moved in such a way as might be expected to rake the spindle towards the wound; since the egg is quite opaque this operation must be made 'in the dark', and there is no way of being certain that the desired result is achieved. In most cases, in fact, the cleavage continued with little change, but in one egg definitely, and in another less certainly, the extending cleavage furrow bent round so as to cut off a small blastomere containing the wound (Fig. 5).

## DISCUSSION

The results even of these preliminary experiments would seem to show that certain theories of cell division which have been proposed in the past are not adequate for the amphibian egg. The experiments under § (i) demonstrate that internal turgor is not a major factor in this form, so that the astral gelation theory put forward some years ago by Gray (1924) for the echinoderm egg cannot apply here. The experiments in § (v) and particularly those in § (vi) make it equally clear that in this egg the cleavage can proceed in the absence of any material connexion between the cortex and the underlying spindle, so that theories which attribute an important role to the contraction of astral rays (such as that of Dan, 1948) must also be rejected.

It appears, in fact, that the formation of the furrow is a function of the cortex itself, since the first pigmented line of the primary furrow can deepen and become properly developed even in a region which has been isolated by means of a cellophane strip. The cellophane is, of course, permeable to small molecules, and it is not excluded that a diffusion of some substance from the spindle plays a part in the deepening of the furrow, but it appears rather doubtful whether diffusion would be rapid enough in the time available.

The capacity to form the furrow would seem to be fixed in the cortex some time before the furrow is actually visible. In the experiments in § (iv), for instance, the furrow appeared in lateral regions which were separated from the first primary furrow by transverse cuts.

The only evidence, in the present experiments, suggesting an influence by the spindle on the cleavage furrow is that of the few eggs described in § (vii) in which an attempt to move the spindle was followed by an alteration in the course of the furrow. General considerations derived from a study of the cleavage of eggs of many groups certainly suggest that the location of the spindle ultimately determines the position in which the furrow appears. These observations do not make it clear whether the influence of the spindle is exerted at the time the furrow is growing, or some time earlier. If further work confirms the present indication that the furrow deviates from its normal course if the spindle is moved during furrow-growth, one will have to conclude that the influence of the spindle is an immediate one. This can be reconciled with the evidence of the cellophane strip experiment either by supposing that the spindle's influence is mediated by an easily diffusible substance, or by supposing that it determines only the very early stage of the development of the furrow, which then has a certain autonomy in its growth both in depth and in length.

The few cinema films which have so far been taken with adequate definition to show the granules in the surface of the egg make it clear that rather extensive movements of the cortex occur both just before and during cell division. It seems probable that these play an important part in the mechanism of cell division, which can hardly be understood until they have been elucidated. Further work to this end is in progress.

SUMMARY

1. In experiments on eggs of the newt, *Triturus alpestris*, it was shown that the first cleavage is carried out normally when the internal turgor is completely relieved by the removal of a large fraction of the internal cytoplasm.

2. The furrow can extend and deepen when separated from the deeper parts of the interior of the cell by a strip of cellophane. Thus little or no part can be played by the contraction of astral fibres attached to the cortex. It seems probable that the factors immediately involved in the deepening and extension of the furrow are located in the cortex itself.

3. The bearing of these observations on some current theories of cell division is briefly discussed, and attention is drawn to the probable importance of general cortical movements, which have been detected but not yet analysed.

REFERENCES

DAN, J. C. (1948). On the mechanism of astral cleavage. *Physiol. Zoöl.* **21**, 191.

FANKHAUSER, G. & MOORE, C. (1941). Cytological and experimental studies of polyspermy in the newt, *Triturus viridescens*. II. The behaviour of the sperm nuclei in androgenetic eggs (in the absence of the egg nucleus). *J. Morph.* **68**, 387.

GRAY, J. (1924). The mechanism of cell division. I. The forces which control the form and cleavage of the eggs of *Echinus esculentus*. *Proc. Camb. Phil. Soc.* **1**, 164.

HOLTFRETER, J. (1943). Properties and functions of the surface coat in amphibian embryos. *J. Exp. Zool.* **93**, 251.

SCHECHTMAN, A. M. (1937). Localized cortical growth as the immediate cause of cell division. *Science*, **85**, 222.