THE MECHANISM OF CELL DIVISION IN THE CLEAVAGE OF THE NEWT'S EGG

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(With Plates 1 and 2)

CONTENTS

INTRODUCTION

Early work upon the nature and mechanism of the fundamentally important biological process of cell cleavage has been very adequately reviewed by Wilson (1928). In this book a broad distinction was made between 'cleavage by constriction', which is characteristic of cell division in higher animals, and 'cleavage by cell plate formation', characteristic of higher plants. In the former the furrow between the daughter cells may be seen to grow inwards from a peripheral zone, while in the latter the cell plate may be seen to form first inside the cell at a point intermediate between the daughter nuclei (e.g. Fujii & Yasui, 1954; Davis, Wilkins, Chayen & La Cour, 1954). In both cases the plane of cleavage is approximately at right angles to the spindle axis and across its largest cross-section. Indeed the idea that true cleavage is dependent, for its existence and location, upon a chromosome-bearing spindle (Boveri, see Wilson) is one of the very few early ideas which most subsequent work has tended to support rather than oppose. Boveri indeed showed (with invertebrate eggs or egg fragments) that centrioles with asters may multiply by periodic division in the entire absence of nuclei, chromosomes or spindles, and in these cases some irregular furrowing of the cell surface may occur. Such furrows usually do not penetrate far into the egg and fail to persist, so that Boveri did not regard them as valid examples of cleavage. On the other hand, cases may be quoted where the position of the cleavage furrow was modified by shifting the position of the anaphase spindle by mechanical deformation of the cell or by micromanipulation (Carlson, 1952; Waddington, 1952; Wilson, 1928, p. 159).

Instances of nuclear division occurring without cytoplasmic division are fairly common in nature, where they lead to multinucleate cells. For example, the first twelve nuclear divisions in the embryonic development of *Drosophila* are without cleavage (although centrioles, asters and spindles are present), after which the entire blastoderm cleaves synchronously, the cell walls growing inwards from the periphery. In the majority of cases, however, cleavage regularly follows nuclear division, as if a coupling mechanism exists between the two processes, which in certain circumstances does not operate.

Certain of the more recently advocated theories of cleavage still assign a dominant role to the asters. Gray (1924) considered that astral growth caused cleavage. Dan (1934) and Dan & Ono (1954), in order to explain their observations of surface movement in cleaving echinoderm eggs, consider that expansion of the spindle remnant pushes the asters against the polar surface to cause expansion there, while simultaneously pulling against the furrow surface to cause a local shrinkage. Yet cleavage has been shown to take place in nature in the absence of asters (cf. the study of pollen forming divisions in magnolia by Farr, 1918, and the cytology of coccids by Schrader, 1948), and may occur after the asters have been made ineffective by experimental procedures such as mechanical stirring (Chambers, 1938; Mitchison, 1953) insertion of a cellophane strip (Waddington, 1952) and the application of colchicine (Swann & Mitchison, 1953).

Very recent theories favour the view that cleavage is effected by expansions and contractions of the cell cortex. Dan (1954), while maintaining his original view of cortical contraction in the furrow region at the beginning of cleavage, asserts that the increase in cell surface, produced by cleavage, is finally accomplished by a local stretching or expansion of cortex in the furrow region. If the hyaline layer has previously been removed using calcium-free sea water this is a post-cleavage event. Swann & Mitchison (see Mitchison, 1952, 1953), again for the sea-urchin, consider that cleavage is initiated by a substance emanating from the daughter groups of anaphase chromosomes, which causes a wave of expansion in the cell cortex beginning near the spindle-poles and spreading to the equator where the furrow is formed by an active expansion of cortical material. The views of these authors are now slightly modified (see Mitchison & Swann, 1955).

Another explanation of cell cleavage is provided by Lewis (1939) and Marsland (1939, 1951), who maintain that cleavage is due to the contraction of a ring of gelated material in the furrow region.

In spite of the universal nature of cell cleavage, by far the majority of experimental studies have been made on early cleavage stages in echinoderms; and among these studies, work done after the hyaline layer has been removed by the use of calcium-free sea water has formed the larger proportion. Echinoderms undoubtedly offer a fine experimental material, but there would seem to be a need now for detailed studies to be made on different materials, so that a subsequent comparison of results may allow one to establish which are the fundamental characteristics of all cleavages, and which are peculiar to particular classes of cleavage. Thus cleavages in plants and animals might be compared with profit, as might holo-

blastic, extreme meroblastic cleavages, and intermediate types (see Nelsen, 1952). Cleavages in different types of animal tissues might also be compared with those of the egg.

MATERIALS AND METHODS

The early cleavage divisions of the newt egg offer several features of great value in any experimental study. The first is that of size. The *Triturus alpestris* egg is just over 2 mm. in diameter. This makes easier micromanipulation procedures (e.g. Waddington, 1952) and physical measurements. Secondly, the cleavages take place quite slowly (e.g. the first cleavage division in *T. alpestris* takes about 40 min. at 18° C.), which makes it easier to record the details of a particular cleavage on ciné film, and to perform measurements at intervals during its progress. Again, the newt egg possesses dark brown pigment granules embedded in its cortical layer by which surface movements may be followed. Lastly, the jelly capsule and vitelline membrane are easily removed by manipulation with fine forceps without any damage to the egg itself. It is always an advantage to be able to remove these membranes easily without having to resort to enzyme solutions, calcium-free media and the like, all of which may injure the egg surface; when observations are made with the aid of such media, it is always necessary to demonstrate that any conclusion drawn holds also for normal cleavage. The one-tenth strength buffered Holtfreter's medium is well established by many embryological studies as most suitable for the maintenance of natural development. The material has one major drawback. The large proportion of yolk in the cytoplasm renders it optically opaque and makes it impossible to parallel the many beautiful studies made on echinoderm eggs with polarized light (e.g. Swann, 1951*a*, *b*; Mitchison & Swann, 1952).

One material was used throughout the work described in this paper; that is the first few cleavages in the naturally fertilized egg of *T. alpestris*, studied in a medium of one-tenth strength Holtfreter's saline with a phosphate buffer at pH 7·0.

The more obvious features of cleavage in the newt are described in a large number of works on descriptive embryology. The cleavages are holoblastic. The first two are meridional and divide the egg into four equal blastomeres. They begin at the animal pole (where a slight puckering of the animal surface transverse to the forming furrow may usually be seen), and travel downwards from the pigmented animal surface through the yolky cytoplasm to the vegetative pole.

The analysis of cleavage, presented in this paper, was made possible by the use of three different kinds of experimental approach. In the first place measurements were made on ciné films taken by time-lapse microphotography by Mr Eric Lucey of this department. From these, the changes in shape of eggs during cleavage were followed, and the movement of the surface pigment granules was also recorded. Secondly, the changes in the elastic properties of the cortex were measured using an apparatus similar to that devised and used previously by Mitchison & Swann (1954*a*, *b*) for similar measurements with sea-urchin. Thirdly, permanent stained serial sections of eggs at particular stages of the cleavage process were examined

for morphological changes in the interior of the egg. Finally, a theory is presented to provide an explanation for all the observations, and to show how cleavage takes place.

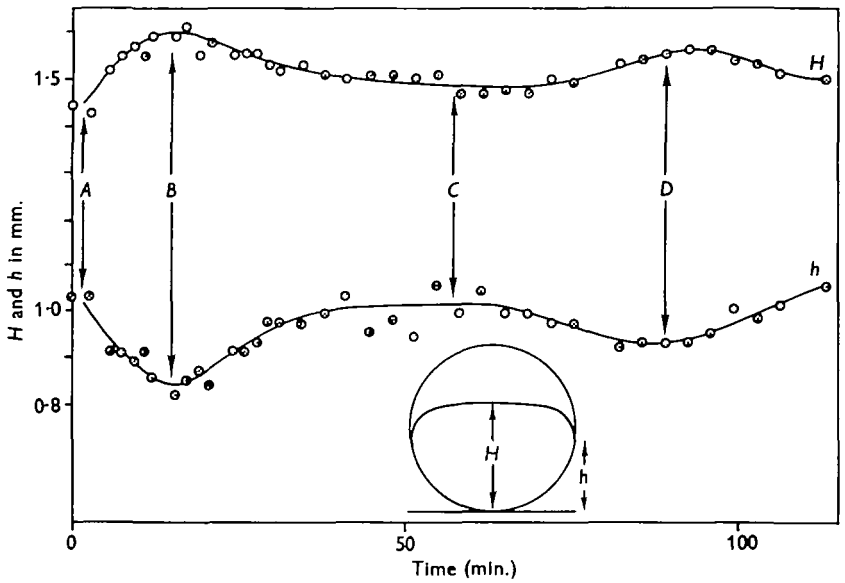
CHANGES IN SHAPE OF THE NEWT EGG DURING CLEAVAGE

When the jelly capsule is removed from a fertilized newt egg before cleavage, the egg may be clearly examined or photographed through the thin transparent vitelline membrane. The undamaged vitelline membrane is almost perfectly spherical. (In fact it is an ellipsoid whose vertical axis of revolution is about 92 % of any horizontal diameter.) The egg itself occupies only the lower two thirds of the intra-vitelline space (Text-fig. 1), so that whereas the vegetative surface is constrained to follow the almost spherical shape of the vitelline membrane, the animal surface, which is always uppermost, shows a pronounced flattening due to gravity. The surfaces of both the egg and the vitelline membrane are smooth. Free movement of the egg surface is not apparently restrained by its contact with the inner surface of the vitelline membrane, as may be demonstrated by inverting the egg within the vitelline membrane, after which it rights itself in a few seconds. After complete removal of the vitelline membrane the egg slowly settles down on a flat surface to a rather flat bun-shape, the equilibrium position being reached in about a minute or two (Text-fig. 2). In this the egg behaves like a rather viscous liquid drop bounded by an elastic cortical layer. When the egg has fully settled down, its shape is governed by an equilibrium between gravitational forces acting on the fluid bulk of the egg (which has a specific gravity of about 1·08) tending to flatten it into a disk, and the elastic forces in the surface layers which tend to return the egg to a more spherical shape. Cleavage proceeds normally, within or without the vitelline membrane, the only real point of difference being that a rather greater area of new cortical material is exposed on the surface in the furrow region in the latter case.

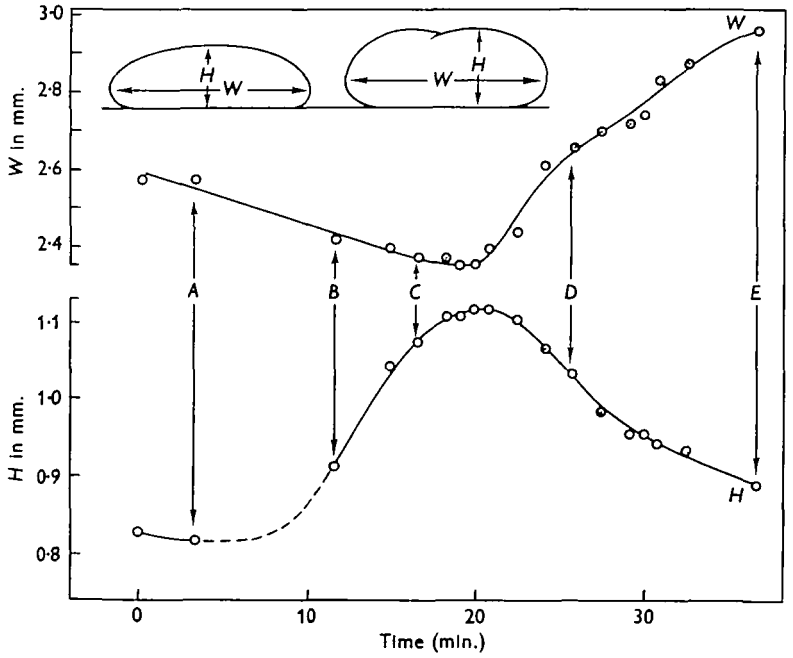
The thickness of the elastic cortical layer is roughly estimated from fixed and sectioned eggs to be 2μ. This estimate is facilitated by examining eggs which have been *lightly* centrifuged before fixation (see later section). Those pigment granules in the more sol-like interior of the egg are then shifted from sites near the animal surface towards the centrifugal pole, whereas those granules embedded in the more gel-like cortical layer remain.

When the surface of an egg is punctured with a needle, it does not crumple like a punctured football bladder (as the internal turgor if any is released). From a small puncture only a slight amount of yolk flows out slowly, and the wound is later closed by a characteristic healing process described by Holtfreter (1943), in which the coat which is the surface layer of the cortex, contracts round the damaged region to seal it off.

Miss H. Yates, of this department, measured the rate of movement of the externally visible furrow across the surface of eggs, using a microscope with micrometer eyepiece and prisms for side viewing. For eggs cleaving within the vitelline membrane, the furrow was found to move from the animal to the vegetative pole at a



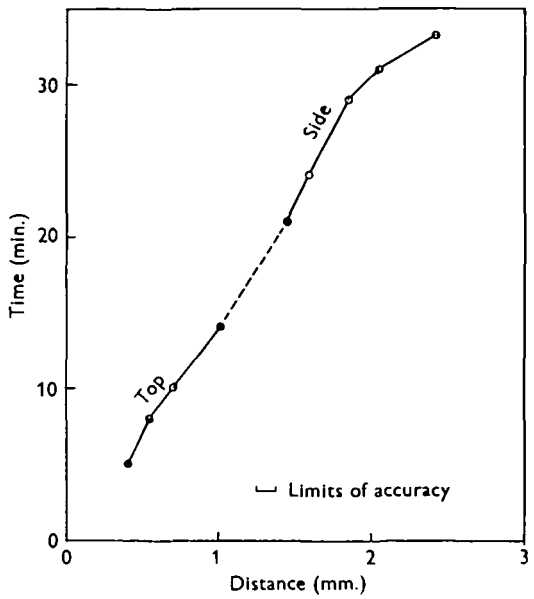
Text-fig. 1. The graphs indicate the changing shape of an egg cleaving within the vitelline membrane. Measurements *H* and *h* (see diagram of egg) were made from ciné film showing the egg in elevation. Times *B* and *D* correspond to the beginning of first and second cleavage respectively. *A* was before first cleavage and *C* midway between first and second cleavage.



Text-fig. 2. The graphs indicate the changing shape of an egg cleaving on a flat agar surface after the vitelline membrane had been removed. Measurements *H* and *W* (see diagram of the egg) were made from ciné film showing the egg in elevation. Times *A* to *B* to *C* were before first cleavage when the egg was 'rounding-up'. At *C* the first sign of the 'dipping-in' at the animal pole was seen. At *D* the furrow was across the animal surface. At *E*, cleavage was complete.

fairly constant rate: the mean value for fourteen measurements was 0·061 ± 0·016 mm./min. during first cleavage at 17·2 ± 0·6° C. The progress of the furrow in a typical case is shown in Text-fig. 3.

Successive frames of ciné films taken to show newt eggs, in elevation, cleaving within the vitelline membrane, demonstrate that the animal surface of the egg reaches a more spherical shape at the moment when the cleavage furrow first appears at the animal pole. The egg subsequently 'relaxes' its shape and is at its flattest roughly midway between one cleavage and the next. This alternate 'rounding-up' and relaxing movement of the egg during cleavage is repeated with each cleavage and



Text-fig. 3. The graph shows the progress of a first cleavage furrow across the animal surface and down the side of an egg cleaving within the vitelline membrane at 17° C.

has been followed on a projected ciné film from first cleavage to the morula stage. Measurements were made of the height (H) of the animal pole of the egg above the vegetative pole, and of the height (h) from the level of the vegetative pole to the level of the contact-point between the side of the egg and the vitelline membrane, using enlarged prints from frames spaced at regular intervals along a film showing a cleavage sequence. The results, plotted against time (Text-fig. 1), illustrate the changing shape of the egg during first and second cleavage.

This behaviour can be observed even more readily in cleaving eggs which are observed in elevation after they have been freed from the vitelline membrane and placed on a flat agar surface. A series of outline diagrams was traced from a film to show the 'rounding-up' process during first cleavage. Again the maximum height of the egg was found to be at the moment when cleavage began, and the movement is shown by a plot of the height (H) and the maximum diameter (W) of the

egg, against time (Text-fig. 2), from measurements made on a film sequence taken during first cleavage.

Harvey & Fankhauser (1933) have interpreted similar observations of changes in shape during cleavage of eggs of the salamander *Triturus viridescens* in terms of 'apparent surface tension'. Using similar methods to theirs our measurements give similar values. However, the egg surface is not like a liquid/liquid interface. The surfaces of eggs contain complicated structural elements (e.g. see Mitchison, 1952), and the behaviour of these when stretched is unlikely to follow the formulations applicable to the surfaces of liquid drops. Although the shape of an egg does superficially resemble a liquid drop, it will be shown in a later section to behave differently under deformation.

The changes in shape of the egg during cleavage are those to be expected from a consideration of the mechanical model of the egg to which we have already referred, If towards the beginning of cleavage there were an increase in the value for the Young's modulus of the elastic cortical layer, or alternatively an increase in its thickness, then the egg would be flattened less easily under gravity and would 'round-up' to assume an equilibrium position more closely approximating to a spherical form.

Using this same model, and assuming the elastic cortical layer to be isotropic, it is theoretically a matter of calculation to derive a value for the Young's modulus of the cortex from measurements on the shape of the egg and using estimates for the thickness and Poisson's ratio of the cortical layer. However, apart from the fact that the cortical layer is almost certainly not isotropic (it would be expected to have different elastic moduli for stresses within the plane of the surface than for directions normal to it), the calculation itself is not straightforward and we have not succeeded in doing it. Fortunately the experimental conditions of the measurements on the elastic behaviour of the cortical layer, described in a later section of this paper, lend themselves more easily to calculations of values for Young's modulus.

One particular film sequence, from which the illustrations in Pl. 1, figs. 1–4, were taken, clearly showed 'rounded-up' positions during cleavage in which the height of the individual blastomeres was greater than their horizontal diameters. Such behaviour cannot be accounted for merely by postulating changes in thickness or elastic properties of the cortical layers; some additional factor is involved. The behaviour is explained by the formation of new cortical material growing out of the cleavage furrow region (see later).

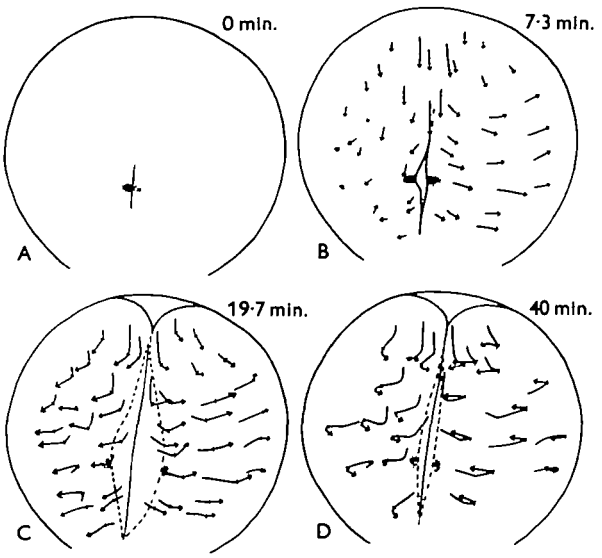
MOVEMENT OF SURFACE PIGMENT GRANULES DURING CLEAVAGE

When a number of film sequences of eggs cleaving within the vitelline membrane were projected, it was found that the clusters of pigment granules on the animal surface could easily be resolved and their movement followed on the screen. By tracing the movement of individual clusters with a pencil during cleavage, using markers fixed at intervals along the film to indicate passage of time, a diagram showing the movement of the entire animal surface of the egg was built up. The pigment granules, whose movements were thus observed, are embedded in the surface

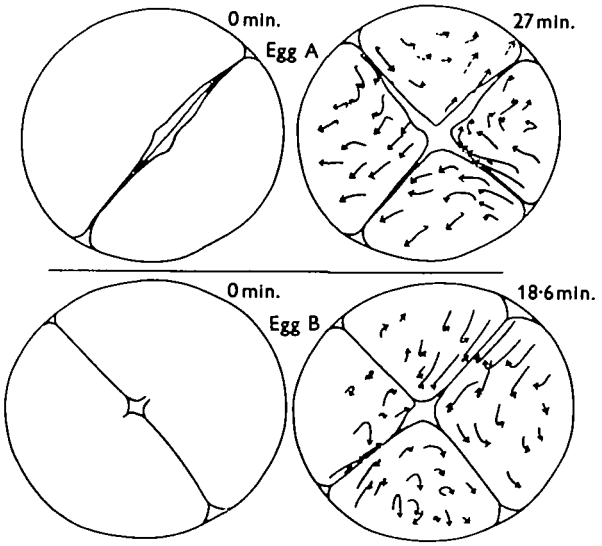
layers of the cortex (i.e. the coat), in gel of considerable rigidity; this can easily be demonstrated by micromanipulation. It is justified therefore to regard the movement of the granules as indicating movement of the surface cortical layer in which they are embedded. During the cleavage sequence the egg rests upon its vegetative surface within the vitelline membrane. Sometimes during the filming, the egg may rotate or rock slightly about its vegetative pole. Such a movement is easily detected and measured by observing its effect upon the position of the outline of the egg or a cleavage furrow. The effect of such a movement of the whole egg upon the observation of relative movements of the pigment granules with respect to each other and the forming furrow, is to complicate the diagram unnecessarily. In such cases, a correction was applied, using a vector method to subtract the movement which each pigment granule possessed as a result of the movement of the whole egg from the total observed movement. It is important to note that this correction does not alter in any way the relative motion of the pigment granules with respect to each other; nor does it alter their movement with respect to the position of the forming furrow. All the illustrations of pigment movement in this paper (Text-figs. 6–8) were taken from film sequences of cleavage in which the correction for the movement of the whole egg was a small or negligible fraction of the total movement observed on the screen. All the movement in the diagrams took place during cleavage; between cleavages little or no movement of pigment granules could be seen.

Detailed diagrams showing the paths of pigment granules during seven cleavages were observed in this way. All showed a similar pattern of behaviour. Cortical movements are seen to be greater near the forming furrow and least on that part of the egg surface remote from the furrow and opposite the poles of the spindle.

When viewing the egg from directly above the animal pole, the beginning of cleavage is seen to be accompanied by a movement of cortex along the line of the future furrow, from the sides of the egg toward the animal pole at which the furrow first appears. This takes place in the first 5–7 min. of cleavage, and it is obvious in all films of first or second cleavage showing the animal surface. It accompanies the 'dipping in' of the new furrow at the animal pole, and the temporary appearance of the tiny wrinkles in the coat transverse to the forming furrow. At the same time, the pigmented cortex on either side of the forming furrow begins a slower movement in a direction away from and at right angles to the furrow. The furrow thus opens out in its widest extent, which is reached about 20 min. after the beginning of first cleavage, when white unpigmented cortex is revealed within the new furrow and the furrow has reached rather more than half way round the outside of the entire egg. The line of demarkation, between the pigmented animal surface on either side of the furrow and the unpigmented cortex within it, is clearly defined (see Pl. 1, fig. 7, and the position of dotted lines in Text-fig. 4*c*). There is no sign of a local cortical movement into the furrow such as Schechtman (1937) has considered to be the immediate cause of cell division in newt; in fact the movement is in the opposite direction. During the final 20 min. in which cleavage is completed, the pigmented cortex on either side of the furrow moves slowly back towards it again, and the white unpigmented cortex within the furrow retreats beneath the surface to form



Text-fig. 4. Four diagrams traced from projected ciné film to show movement of pigment on the animal surface of an egg during first cleavage within the vitelline membrane. In each diagram are shown the complete courses traced out by pigment granules since the beginning of the cleavage. In C and D the distance between the arrow-tip and the mark across the length of the arrow indicates the path traced out since the time of the previous diagram. In B the arrow length indicates the entire path of the pigment since A.

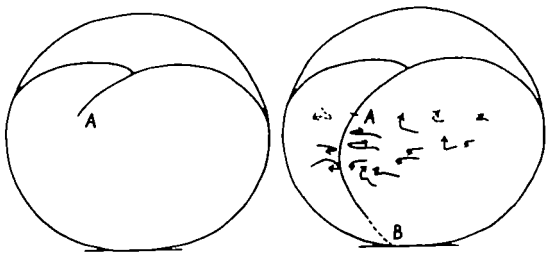


Text-fig. 5. Diagrams of the courses traced out by pigment on the animal surface during the entire second cleavage period for two different eggs cleaving within the vitelline membrane.

part of the cortex by which the daughter blastomeres remain in contact after cleavage.

When an egg is viewed by ciné technique to give a side view of the egg as the furrow moves down to the vegetative pole (see Text-fig. 6), the pigment is seen to move towards the forming furrow, and then away from it. The movement is clearly larger near the furrow and very small on the surface opposite the spindle poles.

The path which any individual pigment granule traces out on the surface of an egg during cleavage is thus largely cyclic, and the distance between the initial and final positions is usually much smaller than the total length of the path traced. Such net translational movement as does take place is in a direction toward the animal pole for regions toward the sides of the egg and in line with the forming furrow; it is away from the furrow for pigmented regions on top of the egg on either side of the furrow, and it is toward the furrow region, for cortex at the sides of the egg nearer the vegetative pole. The translational movements themselves thus make up a



Text-fig. 6. Diagram of surface pigment movement on the side of an egg during first cleavage, while the furrow progressed from the point *A* to the vegetative pole *B*.

cyclic pattern on the surface of the egg, which means that during a cleavage a large change in net surface area of pigmented cortex or coat is not to be expected.

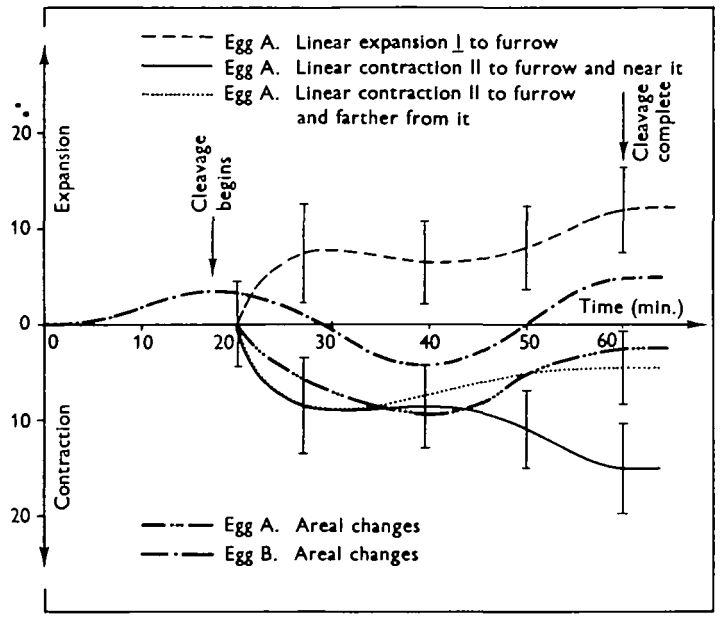
However, it is only fair to test hypotheses of cleavage which depend upon expansions of the cortex which exists before cleavage by measuring linear and areal expansions. This was done for the pigmented cortex on either side of the forming cleavage furrow, where the animal surface of the egg was fairly flat.

The mean percentage changes in distance between pairs of pigment granules were calculated for specific regions on the egg surface, and for measurements made in particular directions across the egg. All measurements were made using the film sequences showing the pigment movement in the greatest detail.

The linear expansions and contractions plotted against time (Text-fig. 7) show the behaviour of the animal surface of a particular egg; these results are means of 144 individual measurements made between pairs of pigment granules. The results for other eggs were similar, in both first and second cleavages. During the entire cleavage process, there is approximately a 10% linear increase of the animal pigmented surface on either side of the furrow for measurements made in directions perpendicular to the furrow. At the same time and place, a 10% linear decrease is observed, when measurements are made in directions parallel to the furrow. The

total linear decrease which takes place is slightly greater than 10% when measured for the pigmented surface adjacent to the furrow, and rather less than 10% when measured along lines further away from the furrow. Areas of the cortex thus change their shape during cleavage, and this has also been shown by staining areas of the cortex with vital dyes.

Areal measurements were made using a planimeter on the prepared diagrams of pigment movement. The areas were outlined by as many pigment granules as possible, and were roughly of equal breadth and width. The areas did not include the unpigmented regions of the furrow, and were chosen to be either on those parts



Text-fig. 7. Graphs of linear and areal changes measured for the animal surface of the egg for which the pigment movements in Text-fig. 4 were recorded.

of the animal surface which were fairly flat, or on the sides of the egg where the curvature did not alter during cleavage because the egg surface was in contact with the vitelline membrane.

The percentage changes in surface area, for pigmented regions on the tops of the two eggs during first cleavage, are plotted against time in Text-fig. 7. It will be seen that the final area does not differ from the initial area by more than 5% in either case.

In Table 1 are shown the percentage areal changes of the pigmented cortex in regions where pigment granules could be used to define areas with sufficient accuracy. Where the areas to be measured are changing shape, it is essential to outline the areas by as many points as possible round the perimeter. If the areas are poorly defined the measurements become very unreliable, and the areal results

of Dan & Ono (1954) for sea-urchin would appear to be open to criticism in this respect. It will be seen from the table, that where measurements show that a significant expansion has taken place on one side of the furrow (e.g. an expansion of the order 10%), then in every case a corresponding contraction will be found to have taken place on the other side of the egg. This behaviour could have resulted from a slightly unequal division of the original pigmented cortex between the two blastomeres. Where measurements were made for areas on each side of the cleavage furrow, an average of the percentage changes was taken. For second cleavages, the average was taken of the measurements made on the animal surfaces of each of the four blastomeres. In no case did this average deviate significantly from zero. There is thus no evidence for any net change in surface area of the pigmented surface during cleavage.

Table 1. *Changes in area of pigmented cortex during cleavage*

Cleavage	Egg	View	Side of furrow	No. of granules round area	% areal changes	Average areal change (%)
1st	A	Top	Left	15	− 7·3	− 2·95
		Top	Right	20	+ 1·4	
1st	B	Top	Left	7	+22·8	+ 4·9
		Top	Right	6	−13	
1st	C	Side	Left	10	+13	+13
2nd	B	Top	Left 1	9	−15·2	− 1·75
		Top	Right 1	11	−13·7	
		Top	Right 2	9	+21·9	
		Top	Left 2	5	0	
2nd	D	Top	Left 1	14	+ 5·9	+ 1·9
		Top	Right 1	8	− 9·3	
		Top	Right 2	11	−21	
		Top	Left 2	10	+32	
2nd	C	Side	Left	5	− 6	− 6
3rd	C	Side	Left	6	+13	+ 1
		Side	Right	5	−11	
					Mean of all eggs	+1·4±5·7%

Now it is known from geometrical considerations that if an egg cleaves to give two equal daughter blastomeres, each of similar geometrical form to the original egg, then a 26% increase in surface must occur. Where the daughter blastomeres do not separate completely but remain with plane surfaces in contact, as in the newt, the increase in surface resulting from cleavage is slightly greater than this. For the first cleavage in the newt within the vitelline membrane, the increase required would be at least 30%, of which all but about 2% would be required to form the wall between the cells beneath the surface of the egg.

This large increase in area of cortex, to be provided during the first cleavage, is only to be reconciled with our conclusion that the total area of the original pigmented cortex does not expand at all during cleavage, by assuming that an additional area of new cortex is synthesized during cleavage in a region where we could not

observe it; that is in the furrow region beneath the surface of the egg. The only possible basis upon which we could retain Schechtman's hypothesis that localized growth of the original cortex on either side of the furrow produces cleavage, would be to assume that the growing region was a band round the egg which extended less than 50μ on either side of the furrow, and which increased in area during cleavage at least ten times. It would not be sufficient merely to assume an expansion of this area, because the expanded surface would be then so thin that it could not possibly possess the mechanical rigidity demonstrated in the next section. Presumably this enormous increase in area would so dilute the pigment that it would appear white when it subsequently appeared in the furrow region 20 min. after cleavage had begun. Our interpretation will be seen later to be rather different from this.

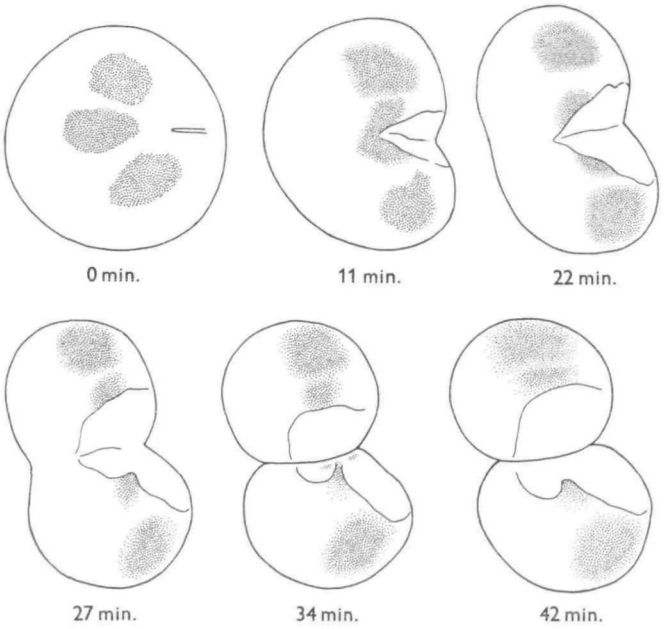
When newt cleavage takes place on a flat surface with the vitelline membrane removed, the increase in surface area is about 27%, but since an 11% increase is sufficient to form the plane surfaces by which the blastomeres remain together, another 16% increase must be provided for the outer surface. The new unpigmented cortex produced in the furrow region is in this case required to cover part of the outside of the egg, and for this reason the unpigmented cortex in the furrow region is considerably more conspicuous. Seen from above, the plan outline of the egg changes from a circle before cleavage to a figure of eight when it is complete. There is thus considerable translational movement of the daughter blastomeres, away from the furrow, as the blastomeres fall apart under gravity with the completion of cleavage. Otherwise the behaviour and motions of pigmented cortex are similar to cleavage within the vitelline membrane. The translational movement considerably reduces the accuracy of any areal measurements which may be made on eggs after the vitelline membrane has been removed.

It will already have been noted that the magnitude of the linear changes between pigment granules in the cortex of newt is incomparably smaller, relative to the dimensions of the egg, than has been observed for cleavage in echinoderms. Dan, Dan & Yanagita (1938) and Dan & Ono (1954) report linear expansions of 50 and 100% between kaolin particles embedded in the cortex on the same side of a forming cleavage furrow, and although the changes are greatest near the furrow, even in the regions opposite the spindle poles linear expansions of 20 and 30% are recorded. The corresponding areal estimates are also very large.

If, using our data for a newt egg cleaving within the vitelline membrane, measurements between pigment granules located on opposite sides of the cleavage furrow are made during the first 20 min. of first cleavage, then indeed linear and areal increases of several hundred per cent may be recorded. Such results would not of course indicate a corresponding expansion or growth of the cortex, since in that period of time the new unpigmented cortex (which as we show later has been formed beneath the surface of the egg) is being progressively revealed in the furrow region as the daughter blastomeres fall apart on either side.

A number of experiments were performed by Miss H. Yates, using a local vital staining method. The vitelline membrane was torn above the animal surface of the egg before cleavage, and strips of agar impregnated with Nile Blue were inserted

and left for about 30 min. so that patches of the surface cortex were vitally stained. During cleavage, the positions of the stained patches were followed by making drawings using a camera lucida. The surface movements, which were recorded by this method, were always similar to those observed from the behaviour of pigment granules, but measurements were not made because the dye showed a tendency to spread through the cortex so that the outline of the stained areas became more diffuse with time. The series of drawings shown in Text-fig. 8 support the conclusion that the white surfaces exposed in the furrow region during mid-cleavage has



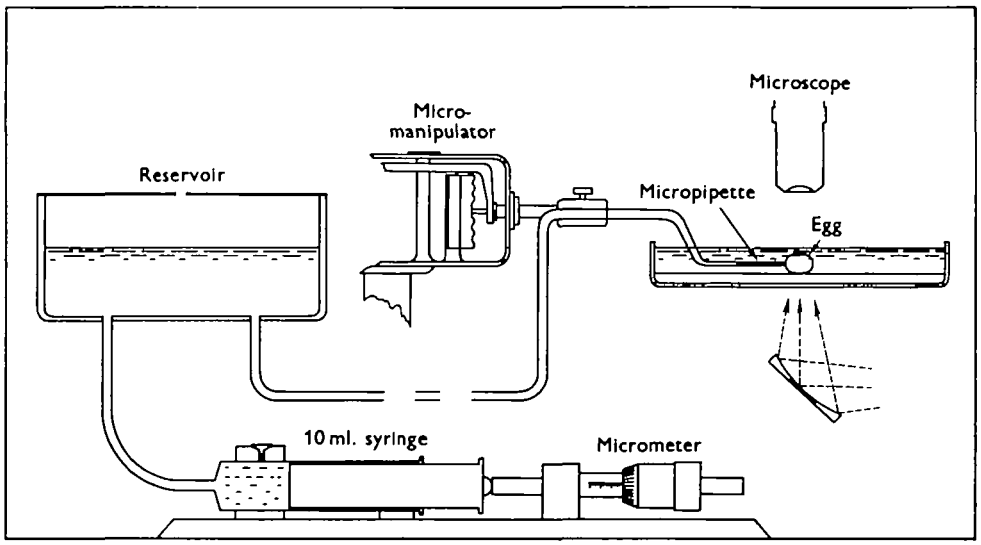
Text-fig. 8. Camera lucida drawings to show the movements during first cleavage of three areas of cortex which had been vitally stained after removal of the vitelline membrane.

been newly synthesized in a subcortical region. The drawings relate to an egg whose vitelline membrane has been removed, so that rather more white surface is exposed in the furrow region on the animal surface than would be the case with the membrane intact. When cleavage is almost complete, a local tendency for a small part of the pigmented coat to spread into the furrow region is shown; this movement is subsequently reversed.

CHANGES IN ELASTIC PROPERTIES OF THE CORTEX DURING CLEAVAGE

These measurements were made using an apparatus and method similar to that previously devised and used by Mitchison & Swann (1954*a*) for measurements on sea-urchin eggs. These authors call their apparatus a cell elastimeter, for it is used to record changes in the elastic properties of the egg cortex. Our form of the

apparatus is shown diagrammatically in Text-fig. 9. After the jelly capsule and vitelline membrane had been removed from the egg, it was allowed to rest on an agar surface in 0·1 strength Holtfreter's saline in a Petri dish upon a microscope stage. The Pyrex glass micropipette was drawn out to give an internal diameter at the tip of 0·24 mm. The wall thickness of the glass at the tip was approximately equal to the internal radius, and the tip was fine ground to give a flat surface perpendicular to the horizontal length of the pipette. Such a surface was found to give an excellent hydrostatic seal when apposed to the side of the egg. The micropipette was mounted on a Fonbrune pattern micromanipulator, and connected by a flexible tube to a cylindrical reservoir of 7·5 cm. diameter in which the saline level

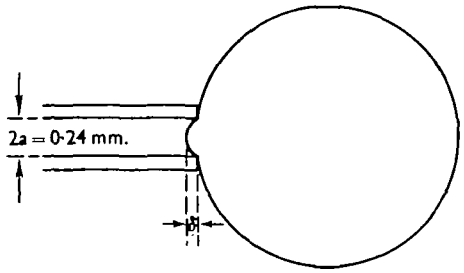


Text-fig. 9. Diagram of the apparatus used to measure the changes in elastic properties of the egg cortex during cleavage.

was approximately equal to that in the Petri dish. Fine adjustment of the hydrostatic level in the reservoir was made using a 10 ml. syringe fitted with a micrometer adjustment.

In making a measurement, the top of the pipette was first placed alongside the egg, opposite but not touching the particular point of the egg surface where the measurement was to be made. Using the micrometer adjustment, the level of the saline in the reservoir was then adjusted until an equilibrium position was reached when water neither flowed in nor out from the tip of the micropipette. The flow of water inside the micropipette was observed by focusing the microscope on minute fat droplets included in the saline. When no flow was observed, the tip of the micropipette was gently pressed against the side of the egg, using the fine horizontal movement on the micromanipulator. Since the diameter of the micropipette was small compared with the diameter of the egg, the surface of the egg then made an

almost flat seal at the end of the tube. The hydrostatic pressure within the micropipette was then reduced a small known amount (p) by withdrawing a small known volume of saline from the reservoir of known surface area. It was then necessary to wait at least 5 min. until the egg surface attained an equilibrium shape within the the pipette. During this time the fat droplets were observed and checked for zero movement. Any movement would indicate a poor seal and the measurement would have to be discarded. At equilibrium the deformation (δ) of the egg surface down the middle of the pipette was recorded using a scale in the ocular of the microscope (Text-fig. 10). Finally the pressure within the micropipette was increased to its former value, the pipette moved horizontally away from the egg, and the zero equilibrium position checked. Any appreciable lack of agreement with the initial equilibrium value, due to evaporation from the Petri dish for instance, would lead to the result having to be discarded.



Text-fig. 10. Diagram of a deformation on the egg surface, produced by a reduction in hydrostatic pressure p within the micropipette, as viewed by the observer down the microscope.

In practice it was found that the flat fine-ground finish of the rather thick-walled micropipette enabled perfectly reliable seals against the surface of the egg to be made without difficulty. In this way, provided the elastic properties of the cortex did not vary during the time the measurements were being made, a series of corresponding values of negative hydrostatic pressure (p) and mid-point equilibrium deformation (δ) could be recorded. The measurements were limited to those values of p for which δ was less than the internal radius of the micropipette. If greater reductions in pressure (p) were applied, the egg was sucked steadily up the tube and was usually destroyed in the process.

When a negative pressure (p) had been applied, during the course of a measurement, the equilibrium value for deformation (δ) was approached exponentially. If, after an appreciable deformation had been produced, the pressure was rapidly increased again to the equilibrium value and the pipette withdrawn, then the shape of the deformed surface was left impressed like a pimple on the egg surface; and thereafter its height also diminished exponentially. The relaxation time, associated with either the decrease or increase of δ, was of the order 3 min. for most eggs before cleavage or after cleavage, provided that the measurement was made on the animal surface or at the side of the egg. At the vegetative pole the relaxation time was rather more than twice this value. Eggs which had been stored in the refrigerator also

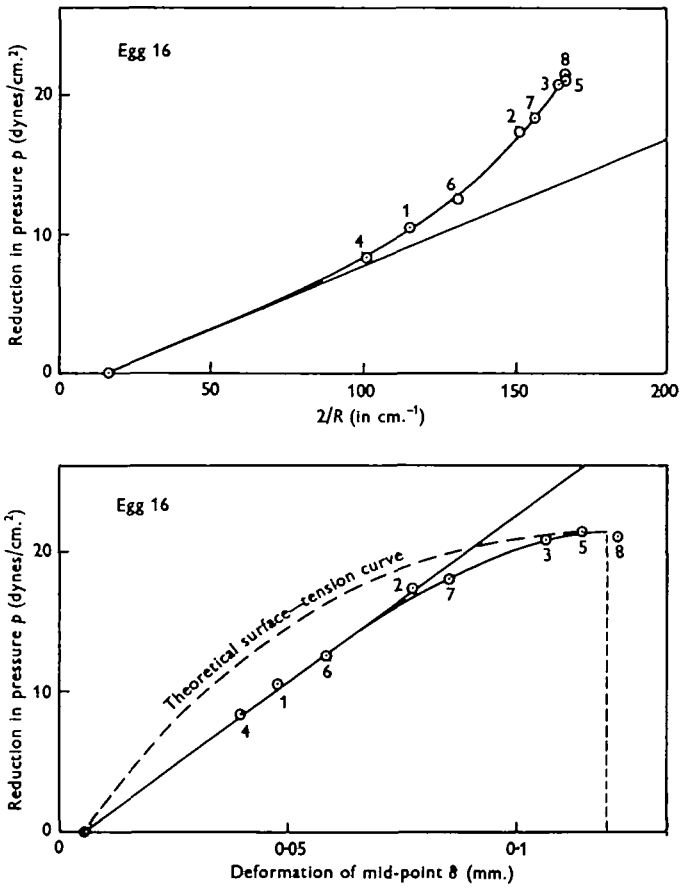
showed large relaxation times. That equilibrium was not attained more quickly, was due therefore to the 'viscosity' of the cytoplasm, which would be greater at the vegetative pole, and would increase on refrigeration. If the cortex possessed plastic in addition to elastic properties, then relaxation effects would again be introduced (Tyler, 1942), but these would not be expected to change towards the vegetative pole. Whatever the contributory causes of the relaxation effect, if the corresponding values of p and δ are to be used to record changes in the elastic properties of the cortex then it would seem essential to use equilibrium values only, even although the rate at which the measurements can be recorded is thereby diminished. Only measurements at equilibrium were used in this study.

Measurements made on the same fertilized egg, between 2 and 3·5 hr. before the first cleavage, are shown in Text-fig. 11. The order in which the equilibrium measurements were made is indicated by numbers. All the measurements lie on a single smooth curve, because during this stage the elastic properties of the cortex do not vary with time. The line is straight for small deformations, but departs from linearity for larger values. This departure from linearity is very probably due to the egg surface slipping round the edge of the micropipette, and this probably does not occur for smaller deformations where the curve is linear. This conclusion may be justified by an experiment in which a large equilibrium deformation is first made (e.g. a value similar to point 4 in Text-fig. 11 is obtained) and the corresponding negative pressure is then halved, without first returning the pressure to zero. When equilibrium has again been attained, this reading will be found to lie below the normal curve (i.e. we have been tracing out part of a hysteresis loop). If the experiment is afterwards repeated, but with the initial deformation on the linear part of the graph (e.g. like point 6 in Text-fig. 11), then the subsequent half value will be found to lie on the straight line and not below it. This behaviour is consistent with a state of affairs in which slipping only occurs over the edge of the micropipette for the larger values of reduced pressure which are sufficient to overcome the frictional forces associated with the coat slipping over the ground glass surface. If these views are correct, then the slope of the linear part of the curve will be proportional to the Young's modulus of the cortical layer of the egg, and also proportional to the third power of its thickness. Measurement of the slope of the linear part of the curve will therefore give a measure of the resistance to flexure on the part of the surface cortex.

If we assume the deformations to be spherical in shape we may replot the observations in terms of the curvature ($2/R$) produced by the applied negative pressure (p) where $2/R = 4\delta/(\delta^2 + a^2)$, and where R is the radius of curvature produced within the pipette of radius a. The resulting curve is shown in Text-fig. 11, and the fact that it is not linear implies that the surface layers of the egg do not behave under deformation like the surfaces of liquid drops. The 'apparent surface tension' concept is therefore inapplicable here. On the contrary, the egg surface behaves like a solid layer possessing elasticity.

It was found that precisely similar values were recorded for measurements made on any part of the egg surface during similar periods with respect to the cleavage

cycle. This result was true for eggs before cleavage, during cleavage and after cleavage. The results did, however, vary with time during cleavage. Measurements were made on fifty eggs during cleavage stages. When the results were plotted as equilibrium deformation (δ) against the negative hydrostatic pressure (p) required to produce it, the results always lay on curves which were similar in form to that

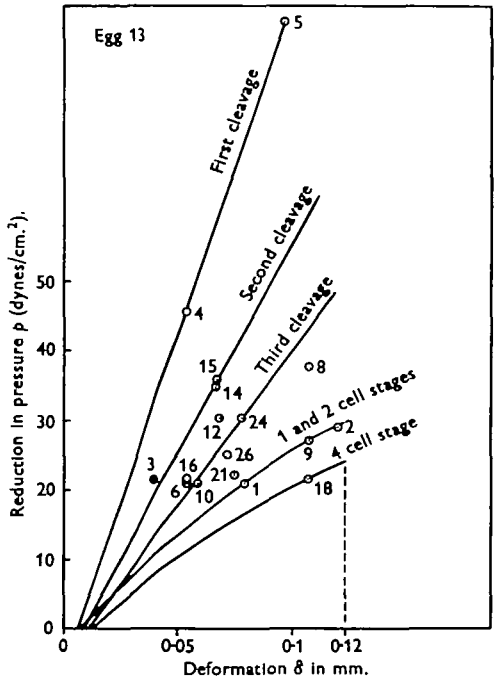


Text-fig. 11. Graphs of mid-point deformation δ and curvature $2/R$, plotted against the reduction in hydrostatic pressure needed to produce the deformation, for a fertilized egg at 18° C. between 2 and 3½ hr. before first cleavage. The numbers on the points indicate the order in which the measurements were made.

shown in Text-fig. 11, but whose slopes varied with time in a regular manner during the cleavage cycle. The curves were however always linear for the smaller values, and the variation of their slopes with time during cleavage was measured.

A series of measurements made upon a single undamaged egg, while it developed at 18° C. from an uncleaved egg until third cleavage, is shown in Text-fig. 12; the order in which the measurements were made is indicated by numbers. The curves which have been constructed are those with maximum and minimum slopes during

the period. Measurements lie on a line of minimum slope for eggs more than 40 min. before the first cleavage and when a cleavage has just been completed. As cleavage approaches, the individual measurements lie on curves of gradually increasing slope, until the moment when cleavage may just be seen to have begun, when they fall on the line of maximum slope. Thereafter until cleavage is finally complete, the measurements lie on lines of decreasing slope. This cycle of events is repeated for each successive cleavage.



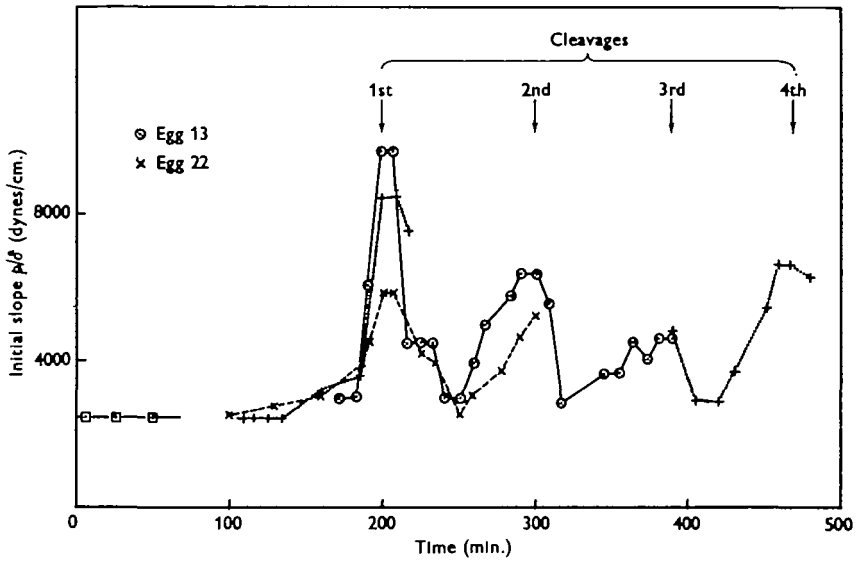
Text-fig. 12. Measurements of mid-point deformation δ and corresponding values of pressure reduction p, for a single egg during its first three cleavage divisions. The numbers indicate the order in which the results were obtained. The drawn curves are those of maximum slope (reached at the beginning of each cleavage), and the minimum slope (between cleavages).

The slopes of the linear parts of these curves were then plotted against time, and the results for the same egg are shown in Text-fig. 13. All cleaving eggs went through a similar cycle of events, but the ratio between the maximum and minimum slopes varied for particular eggs between limits shown in Text-fig. 13, in which measurements for an egg showing the largest variation at first cleavage are plotted together with those for another egg which showed the smallest variation. Values for all other eggs (only one other is shown in Text-fig. 13) lay between these extremes. There was no obvious correlation between the value of the maximum to minimum ratio and the degree of pigmentation of the egg.

Qualitatively then, the mechanical properties of the cortex, as revealed in this experiment, vary with time in a similar manner to that adduced from observations

of the 'rounding-up' of the egg at cleavage: the cortical layers of the egg show maximum rigidity at the beginning of cleavage. These results are also broadly similar to the results obtained by Mitchison & Swann (1955) for cleavage in sea-urchin.

Using the simple elastic shell model of the egg, a doubling in the value of the ordinate p/δ in Text-fig. 13 (which records a rather similar quantity to that which Swann and Mitchison call stiffness) would indicate a doubling in the value of Young's modulus for the cortex if its thickness were known to remain constant, or if, on the other hand, Young's modulus were known to remain constant an increase in thickness of 26% would be indicated. The ratio of the maximum value for p/δ at first cleavage to the minimum value after it, is 3·3 for the egg with the greatest variation and 2·35 for the egg showing least variation. If we assume that during cleavage no



Text-fig. 13. Graphs showing the variation with time during cleavage of the ratios p/δ, measured from the linear portion of curves of p against δ, for eggs at room temperature. The ordinate is proportional to the flexural rigidity of the surface layers. Egg 13 was the egg which showed the greatest degree of variation during the cleavage cycle. Egg 22 showed the least variation.

significant change in Young's modulus for the cortical material takes place, and that no synthesis in the total quantity of cortical material takes place either, but that the existing cortical material is merely distributed by an unspecified process over a larger surface, then taking the values for the eggs showing minimum variation we calculate that the additional surface which could be produced at first cleavage would be 33%, which is in agreement with our previous estimate for the new surface required.

Measurements on the elastic properties of the cortex give similar values during similar periods of time with respect to the cleavage cycle, no matter whether they are made on pigmented cortex near the furrow, on surface opposite the spindle poles, or on animal or vegetal surfaces. Moreover, when a measurement is made

between 20 and 30 min. after the beginning of first cleavage, on the new unpigmented cortex in the furrow region on the animal surface, then here also the value is similar to measurements made elsewhere at the same stage (i.e. the value is slightly above the minimum value). This clearly means that the unpigmented cortex is not the original cortex which has greatly expanded in surface area, for if that were so its small thickness would make it more easily deformable. At the time when the new cortex, having moved out from the furrow region, appears on the surface, its rigidity is as great as the remainder of the cortex.

If, however, the micropipette is deliberately thrust further into the forming furrow, pushing the daughter blastomeres aside in the process, a measurement may be made on surface which gives about half the usual value. This surface is probably either in process of formation or has been damaged in the process of getting the micropipette into position, for at the end of the measurement the pipette cannot be withdrawn easily away to leave an undamaged surface as is normal. In this case the surface adheres to the tip of the micropipette, and long strands of gel stretch between egg and micropipette as the latter is drawn away.

When the furrow begins to move across the animal surface at the beginning of first or second cleavage, the micropipette may be placed for a measurement at the side of the egg in a position which is estimated to be in line with the future course of the furrow. Then an almost hemispherical deformation may be maintained by applying the same pressure reduction which would be required for the micropipette on any other part of the surface at the same time. When this was done, the 'dipping-in' of the furrow eventually occurred quite suddenly to give a sharp cusp in the deformed surface. The shape of the surface then held within the micropipette was consistent with the idea that the initial 'dipping-in' of the furrow is caused by forces (or their resultant) acting towards the interior of the egg from points of application along the line of the forming furrow. One could, of course, give an explanation for this particular result by invoking the theory of Lewis (1939) and Marsland (1939, 1951) but if the furrowing is caused by a contraction of a cortical zone of gelated cytoplasm, then it can be estimated from the shape of the cusp that the zone must be less than 10μ wide.

Using an estimate of 2μ for the thickness of the cortical layer, and putting Poisson's ratio at 0·4, we have calculated (using a formula for the deformation of a clamped end-plate of a cylinder under pressure quoted by Timoshenko, 1940), that the Young's modulus of the cortical material of an egg before cleavage is $1{\cdot}5 \times 10^5$ dynes/cm.^{2}. This value assumes that our deduction that no slip occurs for points on the linear part of the curves of p against δ is correct. It also assumes that the gel is isotropic, which is unlikely if the structures involved here are at all similar to those described by Mitchison (1952). It is, moreover, probable that the inner wall of the gel cortical layer has no definite boundary. There might be a gradual change to the more sol-like properties of the fluid cytoplasm in the interior of the egg, which would make estimates of the thickness of the gel layer unreliable. For these reasons this value for Young's modulus should be regarded as giving the order of magnitude only, and the measurements described in this section are much

more reliable when interpreted in terms of changes in elastic properties or dimensions, than when used to evaluate the precise values of the constants involved. It would appear that the values for the Young's modulus of the cortical layer of sea-urchin egg calculated by Mitchison & Swann (1954*b*, 1955), using model experiments, also involve the assumption that the gel is isotropic like rubber, and in addition it was assumed that the physical conditions under which the measurements with the sea-urchin eggs were performed were exactly those of the model experiments with the rubber balls (e.g. the same degree of slipping over the surface of the pipette occurred in both cases).

An additional series of measurements was made upon cleaving eggs whose cortex was repeatedly punctured with a fine needle so that a little yolk was lost each time. Provided that the egg was not damaged so that it failed to cleave and subsequently degenerated, the measurements which were made under these conditions were those which might have been made upon an undamaged egg. There is here no evidence for any internal turgor within the egg, but it should be remembered, on the other hand, that the newt egg does give an efficient healing reaction to small punctures. It is interesting to note that Swann & Mitchison calculated that the turgor within a sea-urchin egg is either very small or absent, using measurements made using the micropipette on eggs in hypertonic sea water (Mitchison & Swann, 1954*b*, 1955). The egg of the newt is much less permeable to small ions and molecules than the sea-urchin egg, and similar methods could not be interpreted so as to give an estimate of internal turgor. In the newt, too, the thickness and Young's modulus for the cortical layer are sufficient to maintain the shape of the egg, without there being an internal pressure within the egg. There will of course be at least a pressure difference of 15 dynes/cm.^{2} over the cortical layer at the vegetative pole of the newt egg due to the specific gravity of the egg, but such a pressure is negligibly small for most considerations.

Finally some measurements were made with a micropipette whose cross-section was an ellipse with a 2·3 : 1 ratio between the major and minor axes. Measurements were made with the long axis of the pipette arranged latitudinally and then longitudinally on the side of the egg, but similar values were obtained in both cases. We could by this method, admittedly rather a rough one, detect no anisotropy in the elastic properties of the egg for elongations in directions within the plane of the cortical surface being deformed.

CHANGES IN SUBCORTICAL MORPHOLOGY DURING CLEAVAGE

Eggs were allowed to develop within the vitelline membrane until first cleavage, and after the stage reached by the cleavage furrow had been noted they were fixed in freshly prepared Smith's fixative for 24 hr.* Serial sections were subsequently cut at 7μ, with the eggs orientated in such a way that the cutting edge of the knife was always normal to the plane of the furrow. Permanent preparations were made for

* Smith's fixative: 0·5 g. potassium bichromate, 87·5 ml. water, 2·5 ml. 40% formalin, 10·0 ml. glacial acetic acid.

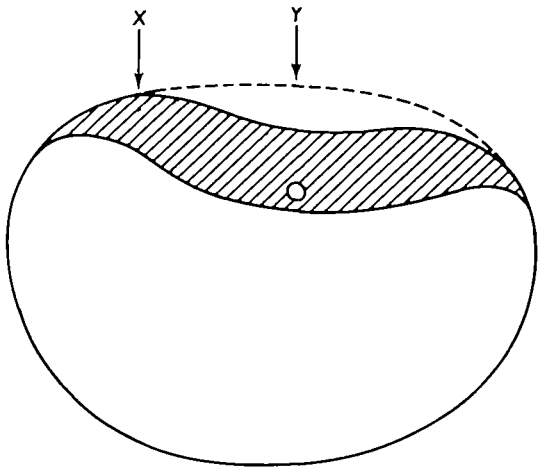
examination under the light microscope after staining with eosin and Delafield's haematoxylin.

In the case of almost every egg there were clear signs of changes occurring in the subcortical cytoplasm in positions below and ahead of the furrow, whose progress could be seen on the surface of the egg. The modified cytoplasm lay only in the plane of the cleavage furrow, midway between the daughter nuclei produced by the mitosis and perpendicular to a line joining the two nuclei. The modifications in the appearance of the cytoplasm which lay ahead of the course of the furrow (Pl. 2, figs. 1, 2 and 4–8) consisted first in a displacement of yolk granules, which in a section produced a line containing fewer or no yolk granules; secondly, in local accumulations of small pigment granules along this same line; and thirdly, in fibres which could be seen to lie across and perpendicular to the plane of the modified cytoplasm.

The displaced yolk was always a feature by which the modified cytoplasm ahead of the furrow could be recognized. Accumulations of pigment, in some particular eggs, outlined the future furrow very clearly, but sometimes the pigment granules beneath the egg surface were rather few, just as the total amount of pigmentation on the animal surface also varies widely between different eggs. When an egg was fixed at a time when cleavage could first be recognized on the animal surface, the fibres were most obvious (P. 2, fig. 1). In the Canada balsam of the mounting medium they also showed positive birefringence. These fibres may be the remnants of the spindle, but they could be recognized in regions slightly to the vegetal side of the nuclei and they extend right up to the animal surface (a distance of about 220μ from a line joining the two nuclei to the animal surface), which would seem to be rather farther towards the animal surface than is usually regarded as normal for a telophase spindle. Spindle remnants can be recognized in the cytoplasm at this stage, however, particularly near the nuclei themselves. They are not found, however, in the yolk immediately on either side of the modified region. The longest fibres which extend across the modified region of displaced yolk are about 25μ long, when the total distance between the daughter nuclei is about 450μ. If the fibres in the region of modified cytoplasm are spindle fibre remnants, it is clear that they have been preserved in this region, whereas to either side they have already degenerated. The wide extent of the region in which fibres are found in the plane of modified cytoplasm leads one to infer that this preservation began when the spindle was at anaphase, for at that time the greatest cross-section through the middle of a spindle is far larger than its value in late telophase. Such a preservation could be understood if the modification in the cytoplasm were interpreted as the formation of a gel layer in line with the position of the future furrow. If the gel layer began to form at anaphase in the plane of the greatest cross-section of the spindle, that is midway between the daughter nuclei, then it is easy to understand the displacement of yolk as the layer of gel thickened, and any spindle fibres would be preserved in the gel, for there they would not be subject to appreciable Brownian motion and would be protected from any streaming movements which might take place in the more fluid cytoplasm. For these reasons, and those to be described later arising from the examination of cleaving eggs after centrifugation, we have interpreted the observa-

tion of modified cytoplasm as due to the formation of gel inside the egg in the path of the future furrow.

From serial sections of a particular egg, fixed when the furrow had begun to move across the animal surface, the extent of the 'dipping-in' of the animal surface along the line of the furrow was measured, and also the depth to which the cytoplasmic modification ahead of the furrow had extended was measured for each section. These results were used to construct a diagram of a section through the middle of the egg in the plane of the cleavage furrow itself, upon which both the 'dipping-in' and the extent of the observed cytoplasmic modifications were recorded (Text-fig. 14). It will be seen that whereas there are always these signs of gel formation below



Text-fig. 14. Diagram of a section through an egg in the plane of the first cleavage furrow, about 8–10 min. after the beginning of first cleavage. The area between the dotted line and the top surface of the egg indicates the extent of 'dipping-in' in the furrow region. The shaded area indicates the extent of the sheet of modified cytoplasm ahead and beneath the externally visible furrow. See also Pl. 2, figs. 1 and 2.

the course of the furrow which would be observable from outside the egg, there are regions (shown on one side of this particular egg) where the gel layer is observed to extend below the surface where no 'dipping-in' of the surface has yet occurred. A section in such a region is shown in Pl. 2, fig. 2. Although there is almost no depression of the animal surface here, for the furrow has not yet reached this part of the egg, yet the line of displaced yolk extending 330μ into the egg is obvious. A line of subcortical pigment granules can also be seen in the modified region (it is clearest about half-way down) with a layer showing displaced yolk $\sim 4\mu$ thick on either side, so that the gel layer appears double. It is important to note that the pigmented cortex at this stage (after the first 10 min. of cleavage) forms an unbroken layer on the animal surface of the egg across the top of the gel layer which extends into the egg along the line of the future furrow. This observation, and the fact that the surface cortical pigment was not observed to move towards and into the line of the future or early furrow when the animal surface of the cleaving egg was

filmed, would seem to rule out any interpretation which assumes that the modified subfurrow region is caused by an expansion or growth of the animal cortex into the line of the furrow. It will be recalled that the movement actually observed was in the opposite direction.

Unpigmented cortex on the animal surface at each side of the furrow, was seen in later sections (Pl. 2, fig. 3) from eggs fixed 20–35 min. after the beginning of cleavage. The implication is that the new unpigmented cortex is synthesized in the region where cytoplasmic modification was observed, and that during mid-cleavage this moves out temporarily on to the animal surface where it was seen in the films of cleavage.

Another group of eggs in first cleavage was centrifuged at between 200 and 400 g. for periods between 5 and 11 min., using a sucrose sugar gradient to support the eggs, which were within their vitelline membranes. The eggs were fixed in Smith's fixative immediately after centrifugation and slides of stained serial sections cut perpendicular to the furrow were prepared as before.

The eggs were not unduly distorted or broken by this rather mild centrifugation. However, some stratification resulted near the animal surfaces of the eggs. In well-pigmented eggs not only is there pigment in the surface layers of the cortex but there is a considerable accumulation of pigment immediately below it so that the total surface layer of pigment which can be seen in a section may be fully 5μ thick. The centrifugation caused displacement of a large part of this pigment from the immediately subcortical layers below the animal surface towards the centrifugal pole, but the surface pigment in the cortex was not moved (Pl. 2, fig. 7). In the furrow region, however, no stratification was ever seen, and the subcortical pigment was not displaced at all. This may have been due to the rigidity of the subcortical gel beneath the furrow, but the furrow in the case of most eggs fixed after centrifugation was rather too far advanced to make this conclusion certain. (Earlier stages of cleavage are difficult to examine after centrifugation because of the time which is spent in the centrifuge.) Nearly all the sectioned eggs fixed after centrifugation showed clear signs of modified cytoplasm extending into the interior of the egg ahead of the cleavage furrow, and in several cases this was bent or distorted, presumably as a result of the centrifugation (Pl. 2, fig. 8). That the modified cytoplasm can be bent about without being destroyed seems clear confirmation of its rigid gel properties. In several instances, moreover, the pigmentation in the modified region formed two lines of granules at a separation of about 4μ, and this suggests that the new layer of gel may already itself be double.

THE INTERPLAY OF THE VARIOUS FACTORS

It is possible to link together all the known facts about cleavage in the newt by forming a comprehensive theory from the deductions we have already made:

From a time which may vary for particular eggs from 20 to about 50 min. before the first cleavage furrow begins at the animal pole of the egg, the cortical layer begins to become a more rigid structure. This causes the egg to 'round-up' and

assume a more spherical shape as it becomes less easily flattened under gravity, and the surface also becomes less easily deformable in experiments with the micropipette. By the beginning of cleavage this change has reached its maximum value, and the structural layer of cortical gel may be assumed then to have increased in thickness by between 30 and 48% if we assume no change in its elastic moduli, although an increase in the value for Young's modulus (of between 130 and 230%) would also give the same result.

Beginning at the time of late anaphase of the nuclear division, new cortex begins to form inside the egg in a plane defined by the mid-points of the spindle fibres (i.e. in the plane of the future furrow). The new cortical gel grows in extent from the animal region, where the spindle is located, and extends gradually into the interior of the egg, towards the side of the egg, and towards the vegetative pole. Signs of the new cortical gel are seen in the sectioned eggs, and there are indications that the gel layer being formed is double. When the new gel has increased in area to about one-sixth of the cross-sectional area of the egg, extending downwards from the animal polar surface, it contracts. Now the upper edge of this area of newly formed gel is an arc which follows the line of the animal surface, and it is joined to the animal surface along this line, so that a contraction of new subcortical gel results in a contraction in the length of its upper boundary also. This contraction was recorded in the analysis of film sequences (Text-fig. 7); it manifests itself in the temporary surface wrinkles on the coat transverse to the first signs of the furrow; it also causes the movement of surface pigment towards the first signs of the furrow from regions in line with the future course of the furrow at the side of the egg, where it is free to move because the new subcortical gel has not yet formed beneath it. The area of subcortical gel continues to increase by growth at its lower edge and the subsequent contraction which occurs later is considered as a phase in the formation of new cortex inside the egg. When its area is about two-thirds that of the total section of the egg, the contraction merely causes a 'dipping-in' as the externally visible furrow reaches to the sides of the egg. At about this time the new unpigmented cortex immediately below the animal surface is fully formed and is a double layer. The coat along the top of the furrow then parts in two, and as the daughter blastomeres tend to fall apart under gravity, the furrows open out to expose new unpigmented cortex on the egg surface in the furrow region, the junction between the old pigmented cortex and the new unpigmented cortex being quite obvious. This is about 20 min. after the beginning of first cleavage. The subcortical gel layer finally grows to meet the vegetal surface of the egg and its subsequent contraction helps to pull the new unpigmented cortex on the animal surface back into the furrow region. When the double layer of new cortex, formed entirely within the egg, is complete in the vegetal region also, the old surface cortex parts, and since it has already joined with the new, the two daughter blastomeres are then fully separate.

The movements and expansions of the pigmented cortex on the surface of the egg, which were described in an earlier section, are always those which might be expected of an elastic surface shell while the new subcortical gel is being synthesized in the manner described above. Its final surface area is unchanged. The additional

surface area produced by cleavage is entirely supplied by new unpigmented cortex synthesized inside the egg and forming those boundary surfaces by which the blastomeres remain in contact after cleavage (and with the slight increase in surface area of a few per cent required on the surface which is externally visible, also supplied by unpigmented cortex visible after cleavage in the furrow region). All cleavage subsequent to the first takes place similarly. The result is that when a morula and later a blastula is formed, the total surface area of the pigmented cortex still has not increased and it still lies on the outside of the blastula, while the inner and contact surfaces of the cells are of the newer unpigmented cortex. The sum total increase in surface area of all the blastomeres is thus in the form of unpigmented cortex. This is in accord with observation.

The manner of the formation of new cortex in the interior of the egg has not so far been considered. We have deduced that gel is forming ahead of the furrow in a double layer. The decrease in rigidity of the entire surface cortex takes place during precisely the period in which the new sub-cortical gel is forming. Some transfer mechanism is indicated. Simple expansion of the surface of the original cortex does not take place; if it did, the movement of pigment would have been obvious. If simple expansion of the inner part of the pigmented cortex was possible while leaving the surface layers at rest, then the observations made on surface pigment could be met, but in that case our deductions concerning the spindle fibres and the forming subcortical gel are certainly false and we would expect to see no fibres in the new subcortical gel. It seems better to assume that the transfer implies a change from gel to sol at the inner surface of the pigmented cortex during cleavage, and from sol to gel along the lower edge of the new unpigmented cortex being formed ahead of the furrow. Such a mechanism implies analogies between amoeboid movement and cleavage, and also suggests that streaming movements in the more fluid cytoplasm take place. Both these suggestions have been made by previous authors (e.g. Davson, 1951; Marsland, 1950), but it is difficult to see how streaming might be detected in newt. However, streaming movements of the general form suggested by Spek (1918, see Wilson, 1928), and observed by him in other species, would seem to be of a kind which would support the above hypothesis, since the flow is round and under the surface of the blastomeres and then towards the furrow region.

That the cortical gel is first synthesized before cleavage, as an additional layer beneath the pigmented cortex already existing, and then transferred to the required region during cleavage, is in line with a number of recent cytological discoveries in which the period before mitosis is revealed as a time of active chemical synthesis, and the mitotic period itself is one of active movement and transport with the minimum synthesis of new materials.

DISCUSSION AND COMPARISON WITH CLEAVAGES IN OTHER FORMS

The most obvious difference between the cleavage process as described here for the newt, and as observed by other authors for the much-studied echinoderms, is that very large surface expansions of the original cortex apparently take place in the latter.

After the hyaline layer has been removed, the first part of the cleavage process in sea-urchin results in the formation of two spherical daughter blastomeres apparently entirely covered by the expanded former cortex. In species with thin hyaline membranes (e.g. *Astricylpeus manni*) the cortex behaves similarly in the natural state; in species with thicker hyaline membranes (*Mesipulus globulus*) the movement of the cortex is inhibited in all except the furrow region (Dan *et al.*, 1938). Measurements of this expansion process were usually made between kaolin particles along the circumference of the largest optical section of the egg, after removal of the hyaline layer (Dan, Yanagita & Sugiyama, 1937; Dan *et al.* 1938; Dan & Dan, 1940). The measurements show linear increases ranging in value from about 10% opposite the spindle poles to about 100% in the furrow region, and with an average value which can be computed graphically to be about 40%. Yet the total increase in surface area possible is only 26%. The measurements of large linear expansions along the largest optical section of the egg then clearly imply that linear contractions are meanwhile taking place in directions at right angles, that is for directions along the surface around the axis of rotation of the egg. Linear measurements in such directions have never been reported to our knowledge. It is calculated that for linear measurements along the surface in such directions an average contraction of about 10% should be found; no difference between linear measurements made in different directions would be expected in the polar region; for subpolar and subfurrow regions linear increases in directions round the axis of rotation would be expected to be appreciably less than for directions along the largest optical section, while in the furrow region itself there must, for directions round the axis of rotation, be not linear expansion, but linear contractions up to about 40%. Such a scheme, deduced from the measurements of Dan and his co-workers, allows the net surface area increase in the first phase of cleavage in echinoderms to be 26% with the hyalin layer absent. Whereas much attention has been given to the expansions in formulating theories of cleavage (e.g. Mitchison, 1952), almost no attention seems to have been drawn to the contractions before the recent paper of Dan & Ono (1954), where areal measurements, made for particular regions of surface, were reported, and an areal contraction was found for the furrow region. Moreover, their figure 12 clearly shows linear contraction along the forming furrow.

A study of the work of Dan (1954*a*, *b*) upon echinoderms which have pigment granules uniformly distributed round the surface of the egg before first cleavage, leads one to infer that the net result of cleavage in newt and sea-urchin is very similar. Here also the pigmented surface remains on the outside while new unpigmented cortex forms the boundary by which the blastomeres remain in contact after a division inside the hyaline layer. In the subsequent blastula stage the pigment is still on the outside surface of the larva (Motomura, 1935). It is also a point of similarity with the newt that the new unpigmented cortex appears to grow in a region midway between the daughter nuclei, but in cleavages studied after the hyaline layer has been removed this event occurs after the blastomeres have been already separated by the process involving an approximately 26% expansion of the original cortex. Here the growth of new cortex appears superficially to be a

post-cleavage event, but its growth nevertheless causes the surface area of pigmented cortex to return to its initial value as in the newt; and so this process is to be regardedas the second phase of cleavage (Motomura, 1950).

It would appear that whereas the cortical movements during cleavage in echinoderms are much greater, when recorded as percentages, than in the newt, yet they do not differ qualitatively except in the matter of timing. The difference between the formation of new cortex in the interior of the egg, as in the newt, or on the surface after the blastomeres have been separated, as in echinoderms cleaving after the hyaline layer has been removed in calcium-free sea water, is merely a matter of the temporal delay of this process in the echinoderms. As regards surface movements, the similarity between newts and echinoderms would probably be closest when the comparison is made with species such as *Astricylpeus* where the spindle is also towards the animal surface.

The correspondence between the general form of the variation in rigidity of the cortical layers during cleavage for newt and sea-urchin has already been noted. Motomura (1950) examined paraffin sections through eggs during cleavage in several species of sea-urchin, and he observed a more lightly staining zone ahead of the cleavage furrow at the beginning of cleavage. There was also an accumulation of granules with specific staining properties round vacuoles in this region, and these structures appeared to Motomura to play a role in the subsequent formation of new surface in the furrow. As described above, we have also found special features in the cytoplasm preceding the appearance of a definite furrow. Thus there is very considerable correspondence in observation and measurement between studies made upon echinoderm and amphibian eggs during cleavage. There is no corresponding similarity, however, between the hypotheses which have been propounded to account for the results obtained.

The ideas which have been suggested here to account for cleavage in the newt do, however, provide a hypothesis for cleavage in echinoderms also. In the first place it is worth noting that for echinoderms as well as newts the various movements of the surface cortex and the linear and areal expansions and contractions are always least at the polar and greatest in the furrow region, with intermediate values in the subfurrow and subpolar regions. The most obvious explanation for this is that the cause of the movement lies in the furrow region. The region of subcortical cytoplasmic modification ahead of the forming furrow was regarded by Motomura (1950) as a region of cytoplasmic weakness, into which the original cortex would presumably expand. He supported this idea by the observation that this region contracted most when the eggs just prior to cleavage were placed in hypertonic sea water. This observation could also have been interpreted by supposing that the dehydration of the region produced by the hypertonic sea water only assisted in the contraction of the modified and gelating cytoplasm which occurs during the first phase of normal cleavage. It is possible that the wave of decreasing birefringence which Swann (1951*b*, 1952) observed to spread from near the centre of each aster outwards to the cell surface, reaching the cortex first opposite the spindle poles, was associated with a progressive solution of the inner surface of the original cortex also

beginning opposite the spindle poles, and this would allow a freer expansion of the surface cortex and also would provide material for the gel which is forming and contracting beneath the furrow. An alternative explanation would be in terms of 'active expansion' of the original cortex involving changes in the folding of protein chains as outlined by Mitchison (1952). This would not, in our view, account for the initial 'dipping-in' of the new furrow, but the difficulty might be circumvented by postulating 'active contractions' in the furrow region itself. In either case the results of the changes in rigidity measured with the micropipette at various points on the cortex of the sea-urchin egg during cleavage will be of interest both during the phase when the original cortex is expanding and subsequently when it is, in our view, being returned to its original area by the formation of new surface in the furrow region. One of the points which future work must settle will be whether the greater percentage linear and areal changes observed in the original cortex of the sea-urchin during cleavage, are primarily due to the cortex of that form being either thinner or possessing smaller elastic moduli than in the newt, or whether the active expansions and contractions of the original cortex are really more important and greater in sea-urchin. It will have been noted that our explanation of cleavage in the newt does not involve 'active expansion' of the cortex.

An objection which is frequently raised against a hypothesis of cleavage which involves contraction in the furrow region is that, when applied to the cleavage of the sea-urchin in a calcium-free medium with the hyalin layer removed, the contracting region must apparently contract away to nothing, for at the end of the first phase of cleavage the daughter blastomeres are connected by the thinnest protoplasmic stalk. In the newt this difficulty does not exist, for the contraction of the original cortex, which we observed on the surface of the egg in the furrow region and parallel to the furrow, is not so great. Furthermore, throughout cleavage in the newt, new gel is being progressively added to form new cortex inside the egg ahead of the newly formed cortex which is contracting. In the sea-urchin, as well as in the newt, it is suggested that before the contraction phase is completed the contracting gel is already double, so that when, in sea-urchins cleaving in a calcium-free medium, the contraction phase finally reaches its fullest extent the daughter blastomeres are seen to be separate. It is noteworthy that both Dan and Motomura have reported that, at this particular stage, the extra granular zone on both daughter blastomeres is noticeably thicker round the base of the stalk, and that the coloured granules (when present) accumulate here also, and both observations suggest a contracted state.

To complete the analogy with the newt, we accept Motomura's conclusion (Motomura, 1950) that new unpigmented surface is formed in this region, where the characteristically staining granules were found to be concentrated. The new unpigmented surface, as in the newt, accounts for the whole of the increase in surface area finally produced by cleavage, and the growth of the new surface returns the pigmented original cortex to its original area, as the areal measurements of Dan (1954*a*) clearly show. Dan (1954*a*), however, apparently believes that the new unpigmented surface is merely the original surface locally stretched, but this could

easily be tested by making rigidity measurements upon it with a micropipette; since the deformability of the surface layer depends inversely upon the third power of its thickness, if Dan is correct it should be very easily deformable in comparison with the pigmented cortex at the same stage. It will be recalled that in the newt no difference in elastic properties of the pigmented and the new unpigmented cortex could be detected.

For cleavages in the higher plants the idea of the formation of a double cell surface, growing from the position of the spindle remnant outwards to join with the original cell surface, seems to fit the published evidence with which we are familiar. It seems possible, therefore, that cleavages in plants may be understood without having to introduce new ideas, but considerably more work remains to be done before one could discuss plant cleavage in any detail. We have no reason to doubt at the moment, however, that cleavages in all plant and animal forms may not take place by processes which are fundamentally similar, in which the points of difference are points of detail due to differences in the relative importance of the many factors involved.

SUMMARY

The early cleavages in eggs of *Triturus alpestris* have been studied.

Ciné-film technique was used to record changes in the shape of the egg and movements of the surface pigment from which measurements of linear and areal changes were made. Local vital staining was also employed. There was no significant net change in the area of pigmented cortex during cleavage.

Before cleavage the egg resembles a viscous liquid drop whose shape is maintained by a uniform elastic shell of Young's Modulus $\sim 1{\cdot}5 \times 10^5$ dynes/cm.^{2} and thickness about 2μ. The egg assumes a more nearly spherical shape immediately before cleavage when the flexural rigidity of the surface layers increases. The flexural rigidity of the cortical layers was found to be maximal at the beginning of cleavage and minimal midway between cleavages. This variation is similar to that previously recorded for cleavage in sea-urchin eggs by Mitchison & Swann (1955), using a similar method. At any particular stage with respect to the cleavage cycle no variation was found in the rigidity at different points on the egg surface.

Serial sections show cytoplasmic modification below and ahead of the forming furrow. It was concluded that the new unpigmented cortex, by which the daughter blastomeres remain in contact after cleavage, is first formed as a sheet of gel (which in later stages can be seen to be a double layer) which grows downwards by a process involving gelation at its lower edge, through the cytoplasm from the animal toward the vegetal surface. The gel layer is assumed to contract immediately after its formation, and in this way to produce 'dipping in' of the new furrow and all the observed surface movements. These ideas have been developed to form a detailed theory of cleavage in the newt, and suggest a common basis for the consideration of cell division in echinoderm eggs, plants and other forms, on the basis that the necessary increase in surface area is achieved by the formation of new cortex rather than by the expansion of the original cell membrane.

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EXPLANATION OF PLATES

PLATE 1

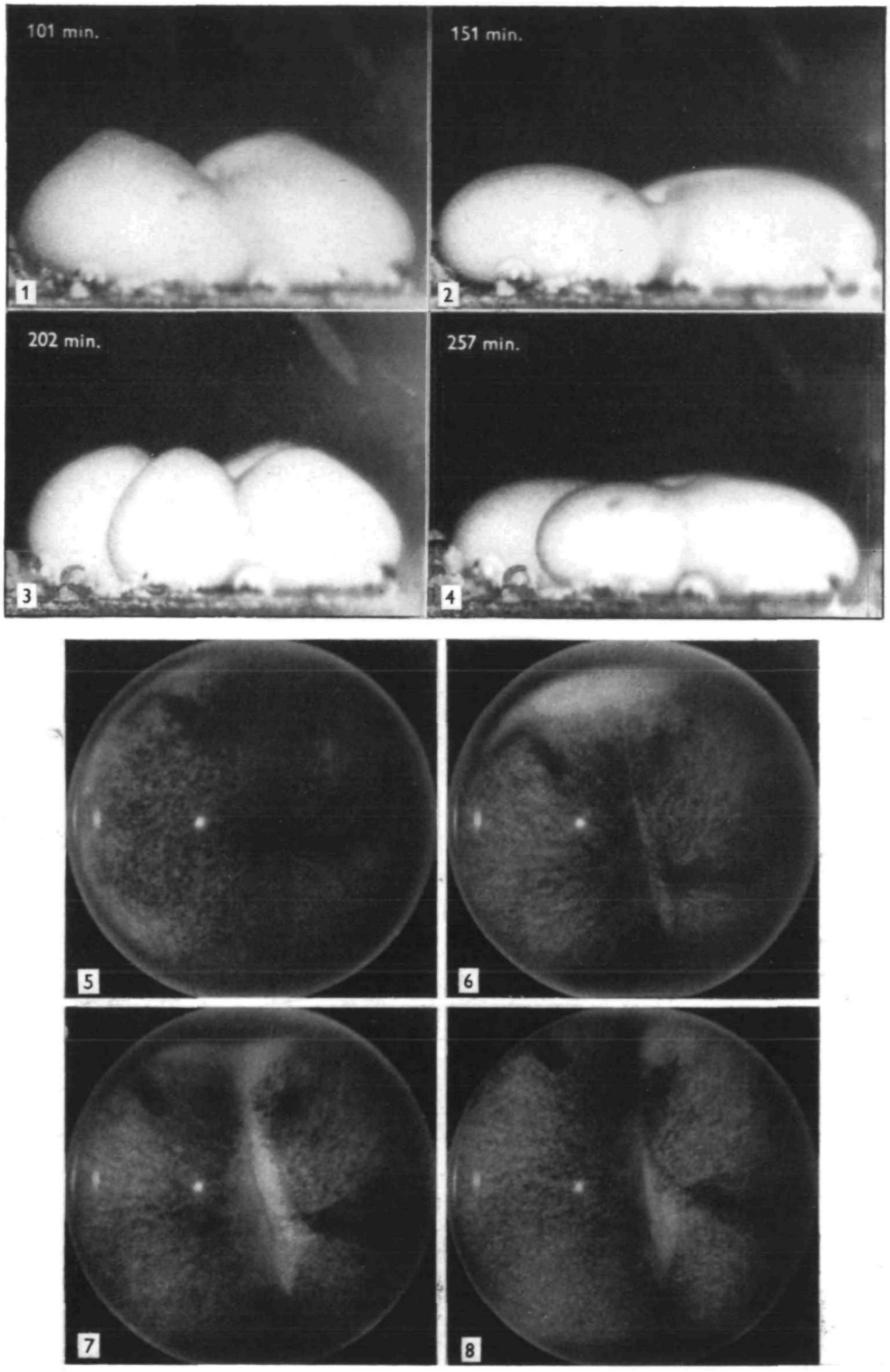
Figs. 1–4. Illustrations from a film sequence of an egg cleaving on a flat surface after the vitelline membrane had been removed. Fig. 1, a 'rounded-up' position of the egg at first cleavage. Fig. 2, a 'relaxed' position of the egg between first and second cleavage. Fig. 3, a 'rounded-up' position of the egg at second cleavage; note that one of the blastomeres is of greater height than breadth. Fig. 4, the subsequent 'relaxed' position of the egg between second and third cleavage.

Figs. 5–8. Egg viewed from above the animal pole during first cleavage within the vitelline membrane, from a film sequence to show pigment movement. A slight rolling movement of the egg about the vegetative pole takes place during cleavage. Fig. 5, 3 min. after the first sign of cleavage; contraction and 'dipping-in' is taking place along the line of the new furrow. Fig. 6, 8 min. after the beginning of cleavage; note the first signs of the furrow opening. Fig. 7, 16 min. after the beginning of cleavage; the early furrow has opened out to reveal an area of new white unpigmented cortex. Fig. 8, 40 min. after the beginning of cleavage. The furrow is closing again, although some white surface still remains in the furrow at the animal pole.

PLATE 2

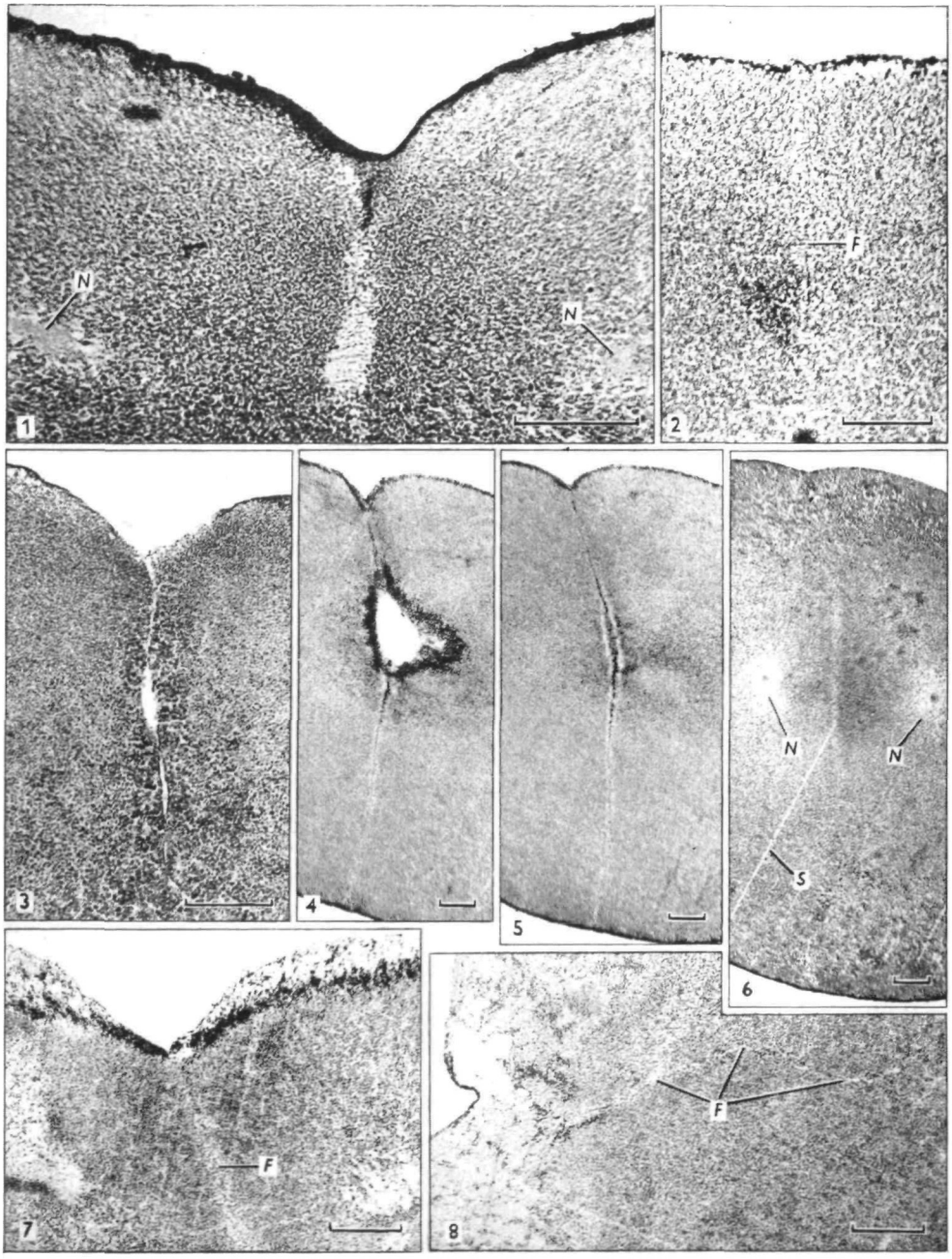
(Microphotographs of sectioned eggs, fixed in Smith's fixative during first cleavage, and stained with eosin and Delafield's haematoxylin. In all cases the drawn scale represents 100 μ.)

Fig. 1. Vertical section cut transverse to the cleavage furrow, through the daughter nuclei *N*, about 8 min. after the first sign of cleavage (see position *Y* of Text-fig. 14). Note the fibres in the area of displaced yolk.



SELMAN AND WADDINGTON—THE MECHANISM OF CELL DIVISION IN THE CLEAVAGE OF THE NEWT'S EGG

(*Facing p. 732*)



SELMAN AND WADDINGTON—THE MECHANISM OF CELL DIVISION IN THE CLEAVAGE OF THE NEWT'S EGG

Fig. 2. Vertical section cut transverse to the cleavage furrow (8 min. after the first sign of cleavage) in a region where the 'dipping-in' of pigmented surface had not yet taken place (see position *X* of Text-fig. 14). Note the line of pigment granules and displaced yolk along the line of the future furrow *F*.

Fig. 3. Vertical section cut transverse to a fully formed cleavage furrow, from an egg fixed 35 min. after the beginning of cleavage. Note that the egg surface at this stage is unpigmented up to about 100 μ on either side of the furrow.

Fig. 4. Section cut in a plane about 10° from the horizontal and transverse to the furrow, at grazing incidence to the 'dipped-in' region of the furrow at the animal pole. Egg fixed about 8 min. after the first sign of cleavage.

Fig. 5. Section cut 40 μ below that shown in fig. 4, from the same egg. Note that the subcortical signs of the future furrow extend right across the egg. Note the line of pigment granules, and that the lighter staining line of displaced yolk is double.

Fig. 6. Section cut 230 μ below that shown in fig. 5 so as to include the daughter nuclei *N*. Note the line of modified cytoplasm midway between the nuclei. The line *S* is an artifact caused by an imperfection of the knife edge. In no case was an egg sectioned so that such an artifact lay parallel to a line of modified cytoplasm.

Fig. 7. Vertical section cut perpendicular to the line of the cleavage furrow across the animal surface. Egg fixed after centrifugation at 1700 r.p.m. for 5 min. after the first signs of cleavage. Note the displacement of pigment and cytoplasmic stratification to either side of the furrow. Note the line of the future furrow *F*.

Fig. 8. Vertical section cut perpendicular to the line of the cleavage furrow across the animal surface. Egg fixed after centrifugation at 1200 r.p.m. for 11 min., after the first signs of cleavage. Note that the line of the future cleavage furrow *F* has been bent by the centrifugation.