ULTRASTRUCTURAL CHANGES IN THE SURFACE LAYERS OF THE NEWT'S EGG IN RELATION TO THE MECHANISM OF ITS CLEAVAGE

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SUMMARY

The surface and cortical layers of an uncleaved newt egg have a characteristic ultrastructure which remains unaltered during cleavage; ultrastructural changes are confined to the region of the furrow. At the onset of cleavage there is a dipping inwards of the rough heavily pigmented animal surface to form a groove. Along the bottom of the groove the surface irregularities are reduced and a dense band (0.1 μm thick and 16 μm wide) is formed immediately below the plasma membrane. Within this band there are parallel filaments, 8-10 nm in diameter, oriented in the direction of the future furrow. No structural modifications were observed below the cortical layers of the leading part of the furrow apart from accumulations of granules and the mid-bodies of the spindle remnant. It is proposed that the dipping-in of the groove is due to contraction within the filamentous band, rather than contraction in a sheet of subcortical gel as proposed previously. The filamentous band persists below the furrow during the later stages of cleavage.

The new unpigmented surface first forms as a strip across the animal surface and begins to grow at the bottom of the groove. Over most of its area, it is much smoother than the pigmented surface and has less material on the outside of the plasma membrane. There are microvilli along the bottom of the groove. The join between the new unpigmented and the old pigmented surface is abrupt. As the new unpigmented surface grows in extent, a narrow furrow forms below the lowest part of the groove and progresses towards the vegetal surface. For most of its length the furrow is between 10 nm and 0.5 μm wide, but at its leading edge it is 2 μm wide with microvilli on its surface and 10-nm filaments below the plasma membrane. It is concluded that the progressive formation of the furrow is due to active growth of new unpigmented cell surface.

At late cleavage a ridge 10 μm high forms at the join between the new and old surface. After cleavage the ridges approach and meet to form the intercellular junction by which daughter blastomeres are held together along the animal surface.

The mechanism of cell cleavage in the newt egg and in other forms is discussed in the light of the present observations.

INTRODUCTION

When mitosis is followed by cytokinesis in plants and animals, the position of the cleavage plane is determined by the position of the mitotic apparatus at anaphase (Wilson, 1925; Zotin, 1964; Rappaport & Ebstein, 1965; Kubota, 1966; Selman, 1966). Once the cleavage process has been initiated, however, it proceeds independently of the mitotic apparatus and involves only the cortical layers of the cell. Hiramoto (1956, 1965) demonstrated this for the sea-urchin egg. Dan & Kojima (1963) showed that
amphibian cleavage can continue in an anuclear fragment excised from the egg ahead of the furrow. Kubota (1966), by observing cleavage after removal of the mitotic apparatus from the frog egg, was able to demonstrate that an autonomous capacity for furrowing developed in the cortex during anaphase.

In a previous study of first cleavage in the newt's egg, Selman & Waddington (1955) recorded the movement of pigment granules in the cell cortex using a time-lapse ciné technique in order to see whether there was an expansion of original pigmented surface into the furrow. Both linear and areal measurements were made using the clusters of pigment as markers. Although a contraction of pigmented surface along the line of the furrow was observed during the first 7 min of cleavage, there was no net change in the area of pigmented surface during cleavage. It was concluded that the pigmented surface does not expand to form the furrow.

Furrow formation in the newt egg is by a progressive growth of new unpigmented white surface, beginning at the animal pole and growing downwards through the egg to reach the vegetal surface. The newt egg is 2 mm in diameter and the cleavage process takes 40 min to complete. During this period the cleavage can be staged by observing the progress of the superficial part of the furrow as it moves round the surface of the egg from the animal to the vegetal pole. The new unpigmented surface, formed in the furrow during cleavage, remains there as the surface by which the daughter blastomeres subsequently adhere to each other.

Modern techniques of specimen preparation can give satisfactory preservation of cytoplasmic details at the ultrastructural level in large yolky eggs. The present electron-microscope observations on cleavage in the newt's egg show the details of furrowing at successive stages. A knowledge of these details makes possible a more complete discussion of the mechanism of cleavage.

METHODS

Uncleaved eggs of Triturus alpestris were collected in pond water and examined using a binocular microscope. Some were decapsulated immediately and others when they had reached the required stage of cleavage. The majority of eggs used in this study were placed in the glutaraldehyde fixative for 1 h before their jelly capsules were removed. This was to prevent distortion since it is much easier to remove capsules from fixed rather than living eggs. The vitelline membranes were left intact throughout the processing in order to protect the egg surface.

The eggs were fixed at room temperature in a 2.5 % solution of glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 6 h, rinsed overnight in buffer containing 3 % sucrose, and post-fixed for 3 h in buffered 1 % osmium tetroxide containing sucrose. They were dehydrated in a graded series of alcohols and transferred through several changes of epoxypropane to an Araldite-Epon mixture. Infiltration was carried out at 50 °C using a rotary shaker (Jurand & Ireland, 1965), after which the eggs were embedded in disk-shaped containers.

For light microscopy 1 μm sections were stained with toluidine blue. Light micrographs were used to locate the field of observation in electron micrographs of adjacent sections. For electron microscopy thin sections were mounted on carbon-Formvar coated grids and double stained with 2 % uranyl acetate and lead citrate (Reynolds, 1963). They were examined in an AEI EM6B electron microscope operated at 60 kV.
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OBSERVATIONS
The pigmented surface and cortex

The surface of the newt egg and the underlying layers of the cortex have a characteristic ultrastructure. The surface is rough with many irregularly shaped projections between 0.2 \( \mu \)m and 0.8 \( \mu \)m high and with a similar width across their base (Figs. 2, 3). The triple-layered plasma membrane follows the surface contours. Immediately outside the plasma membrane there is a layer of loosely packed macromolecular material about 50 nm thick which contains globules about 10 nm in diameter (Fig. 3). Inside the plasma membrane there is a layer of dense granular cytoplasm within which fine fibrillar material can sometimes be distinguished. These fibrils are 5–7 nm in diameter and probably have irregular orientation since they appear for only short distances in any plane of sectioning. The dense cytoplasm lies beneath all the surface projections and is about 0.1 \( \mu \)m thick below the plasma membrane in regions between the projections. A few 30-nm granules occur within the layer of dense cytoplasm and were shown to be glycogen by treatment with periodic acid and lead citrate (Perry, 1967).

Below the dense cytoplasmic layer there is another layer, about 4 \( \mu \)m thick, in which there are many vesicles each 0.1–0.2 \( \mu \)m in diameter, containing only low-density material (Figs. 2, 3). This layer also includes many rounded high-density pigment granules of 0.6 \( \mu \)m diameter, with a substructure of dense particles 80 nm in diameter. There are also glycogen granules of 30 nm diameter. The vesicular layer of the cell cortex probably has a greater rigidity and more gel-like consistency than the interior cytoplasm of the egg since pigment granules are held within it during cleavage and early embryonic development. Some pigment granules are also present in the interior cytoplasm of the egg.

The cell cortex in the vegetal hemisphere of the uncleaved egg has few pigment granules in its vesicular layer but in other respects it has a similar structure. The ultrastructure of the outer surface and cortex in both hemispheres remains substantially similar during and after cleavage, except that certain changes occur in the cleavage furrow itself during its formation.

The internal cytoplasm of the egg includes many yolk platelets, lipid droplets and glycogen granules, some mitochondria and pigment granules, an occasional Golgi complex, but no detectable endoplasmic reticulum except in the immediate vicinity of the nucleus.

Early cleavage—formation of a surface groove

When cleavage begins, the pigmented surface at the animal pole dips inwards to form a shallow groove along the line of the future furrow. At this stage (Fig. 1B) there is only pigmented cortex at the bottom and sides of the groove. In sections cut transversely to the groove, accumulations of pigment granules and yolk platelets were observed below the lowest point of the groove. These accumulations extend to a depth of about 70 \( \mu \)m and to a width of about 15 \( \mu \)m across the plane where the future furrow will form, but no other changes in the cytoplasm were seen in this region. The displaced pigment granules probably indicate a flow of cytoplasm from beneath the
animal surface of the egg towards the bottom of the groove and thence towards the interior of the egg. Only a small proportion of the pigment granules are displaced in this way and the amount of pigment held in the animal cortex appears to remain unchanged.

**Fig. 1.** Stages in the first cleavage of the newt egg. Each drawing is of a median vertical section and transverse to the groove or furrow. In A, the rough pigmented animal surface is shown before cleavage. At the onset of cleavage (B), there is dipping inwards of the animal surface to form a groove. The band of filaments is shown in cross-section. Some displaced pigment is shown below the groove. At early cleavage (C), new smooth unpigmented surface has formed in the groove and there are microvilli at its deepest part. Note the abrupt join between pigmented and unpigmented surface. At mid-cleavage (D), the furrow has grown below the lowest part of the groove. It is widest at its leading edge where there are microvilli and filaments (also see Fig. 9). At late cleavage (E), the furrow approaches the vegetal pole where there are microvilli and dipping inwards has occurred. Note the ridges on the animal surface at the join between pigmented and unpigmented surface. At post-cleavage (F), the ridges have come together to form an intercellular junction. The drawings are not to scale.
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It was noticed that eggs which had been previously decapsulated tended to collapse inwards in the region of the animal pole when fixed at this stage, thus increasing artificially the depth of the groove. In eggs fixed within the capsule this collapse did not occur.

Ultrastructural changes were observed only in the surface layers of the newt egg. In an egg fixed at the earliest stage of the dipping-in process, there were no irregularly shaped projections at the egg surface on a strip of cell surface about 16 μm wide along the bottom of the very shallow groove, and the dense cytoplasm which formerly lay below the bumps seemed to have condensed into a dense layer (0.13 μm thick) under the relatively smooth surface but above the vesicular cortex (Fig. 2). At higher magnifications of the electron microscope, a system of parallel filaments was resolved within this dense layer, both in eggs fixed at the early stage of groove formation and at later stages of cleavage (Figs. 6, 7). The parallel filaments run across the animal surface of the egg in a band of dense cytoplasm below the developing furrow. Measurements showed that individual filaments were between 8 and 10 nm in diameter. Estimates of the minimum diameter of the elliptical profiles of filaments seen in transverse section always gave values in this range. When filaments were viewed longitudinally their diameter appeared to be the same or somewhat less. In this paper the word filament is used only for the thread-like structures in parallel arrays within a band below the lowest part of the cleavage groove or furrow; threadlike elements observed elsewhere are termed fibrils.

When live eggs of *T. alpestris* at the early groove stage of cleavage are observed through their jelly membranes using a binocular microscope, transverse wrinkles in the pigmented surface can be seen to form transversely to the line of the groove. Rugh (1948) illustrated similar wrinkles at cleavage in eggs of *Rana pipiens*. Similar transverse wrinkles are seen in cleaving eggs of *Xenopus laevis* and in this species they persist to late stages of first cleavage. Selman & Waddington (1955) recorded for *T. alpestris* a 15% contraction along the line of the groove during the first 7 min of cleavage (see their text-fig. 4B). Both the transverse wrinkles and the measured contraction suggest the presence of a structure which contracts along the bottom of the groove at the time when the dipping inwards occurs. Following Szollosi (1968a, b) and Schroeder (1968) who both studied coelenterate eggs, it is suggested that in the newt egg the band with 8-10 nm filaments may be this contractile system. The filaments are in the correct position and orientation to produce a dipping inwards of the furrow if they are contractile.

Initial growth of unpigmented surface

At the next stage of the cleavage process, a thin strip of surface with unpigmented cortex appears at the bottom of the cleavage groove. The join between the pigmented and unpigmented surface is quite abrupt, both in a living egg viewed from above the animal surface (Selman & Waddington, 1955) and when an examination is made of serial sections cut transversely to the groove. In one egg, serially sectioned, the surface with unpigmented cortex extended 40 μm on either side of the lowest point of the groove until it joined the pigmented cortex. In other eggs, considerably greater areas of un-
pigmented cortex were exposed in the groove on the animal surface until a stage was reached when the further growth of unpigmented surface took place in a narrow furrow below the lowest point of the groove.

In normal eggs of *Xenopus laevis* the new unpigmented surface appears to form in a narrow furrow below the surface of the egg, even at the earliest stages of cleavage. In *T. alpestris* a similar situation rarely occurs. The origin of the difference appears to be the greater support afforded by the vitelline membrane of the *Xenopus* egg. The extent to which the new white surface is temporarily exposed during cleavage near the animal pole of an amphibian egg seems to depend upon how much space there is between the egg and its vitelline membrane, and this varies with the individual egg, its species and on whether the external jelly capsule has been removed or not. In *Xenopus* there is less space between the egg and its vitelline membrane than in *T. alpestris*. Moreover, if the jelly capsule of the *T. alpestris* egg is removed, an internal turgour within the capsule is released and the colloidal material normally within the capsule is dispersed and diluted. This in turn allows more water to pass through the vitelline membrane to enlarge the perivitelline space, which causes more white surface to be exposed during cleavage. Even more unpigmented surface is exposed should the vitelline membrane be removed.

As soon as the new unpigmented surface appears at the bottom of the groove in cleaving eggs of *T. alpestris*, transverse wrinkling ceases in the pigmented surface to either side. Moreover, transverse wrinkling has never been observed in the unpigmented surface. The non-appearance of wrinkling at this stage may be because the new unpigmented surface is in a state of expansive growth which counteracts any contractility along the line of the furrow.

By electron microscopy, the band of 10-nm filaments has been clearly demonstrated at this stage (Figs. 6, 7). It forms a layer 0.1 μm thick and 15 μm wide between the plasma membrane and the vesicular layer and runs along the lowest part of the groove in the new unpigmented surface.

When sections are cut perpendicularly to the band, adjacent individual filaments are found at a centre-to-centre distance of about 20 nm but the filaments do not appear to be regularly packed. Between the dense filaments there is a matrix of unevenly distributed material of medium electron density.

Microvilli frequently occur on the surface at the bottom of the groove above the filamentous band (Fig. 5), and up to a distance of 30 μm on either side. Microvilli are first observed near the animal pole at the bottom of the groove just before any unpigmented surface is formed, but they are later found there in large numbers on the new unpigmented surface. The microvilli are about 1.5 μm high by 0.3 μm in diameter and contain cytoplasm which includes fine fibrils (about 5 nm in diameter) and a few glycogen granules. Occasionally microvilli are found to be branched.

At this stage unpigmented surface may extend for 250 μm to either side of the bottom of the groove near the animal pole. In such cases, between the microvilli at the bottom of the groove and the surface irregularities of the pigmented surface there is a considerable area of new unpigmented surface which is relatively smooth (Fig. 1c). There is a layer of dense cytoplasm 0.1 μm thick below the plasma membrane of this
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smooth surface (Fig. 4) and this layer has a fibril-granular ultrastructure like that below the surface irregularities of the pigmented surface. There is also much less extracellular material adhering to the plasma membrane of the smooth new surface than is found on the rough pigmented surface (compare Figs. 3, 4). The vesicular cortical layer is present below both new and pigmented surfaces but there are fewer vesicles below the new surface.

Growth of the furrow

The initial growth of new cell surface during first cleavage of eggs of *T. alpestris* is normally, as has been described above, within the open groove which forms across the animal surface, but a stage is reached when cleavage continues as a narrow furrow which forms below the lowest point of the groove. Sections were cut transversely to the groove of an egg fixed at a stage when the furrow first began to go below the egg surface and the electron micrographs showed that the new cell surface on opposite sides of the groove met at the bottom at an acute angle. Here a narrow furrow extended for about a micron below the egg surface. The cell surfaces which were continuous with the sides of the groove were tightly pressed against each other in the short furrow. The band of fibrils and microvilli were also present.

In newt eggs fixed at successively later stages the progress of the furrow was followed in transverse sections as it grew steadily downwards through the cytoplasm (Fig. 1D) until it reached the vegetal surface. In the typical case of an egg fixed when the furrow had grown to a depth of 50 μm below the lowest point of the animal surface in the groove, the furrow was found to be very narrow over most of its length. The gap between opposite surfaces of the furrow varied between 0.5 μm and less than 10 nm (Fig. 8) and so it was difficult to follow its exact course in sections viewed by light microscopy. At the bottom or leading edge of the furrow, however, it widened to a keel-like fold which in cross-section had a bulb-like profile 2 μm wide and 3 μm long (Figs. 8, 9). The cell surface at the wider leading edge of the furrow always possessed microvilli (Fig. 9). Some but not all of these microvilli were more in the shape of surface folds and were wider in the plane of the furrow. The typical microvilli were finger-like protrusions. At the leading surface of the furrow and extending about 7 μm from the tip on either side, there was a dense filamentous layer, 0.1 μm thick below the plasma membrane, and consisting of parallel filaments of about 10 nm diameter (Fig. 9).

Bundles of about 10–30 microtubules, which represent individual spindle fibre remnants, were often observed in the cytoplasm near the cleavage furrow. Individual microtubules were 27 nm in diameter with a low-density lumen of about 10 nm diameter. Midway between the daughter nuclei, the microtubules in each bundle were embedded in a matrix of dense cytoplasm to form a mid-body. The mid-bodies lay in the path of the advancing furrow until the furrow appeared to thrust through the spindle remnants. Before their displacement by the furrow, the microtubules were always oriented perpendicularly to the 8–10 nm filaments. Neither mid-bodies nor microtubules appeared to participate in furrow formation.
**Late cleavage stages**

In the late stages of cleavage the furrow approaches the vegetal pole simultaneously both from above and from either side. The vegetal surface undergoes similar changes to those seen on the animal surface at early cleavage. Thus a groove forms across the vegetal pole in line with the leading edge of the furrow which is 280 μm above it, in the egg's interior (Fig. 18). At this stage the furrow has broken through to the vegetal surface about 250 μm to either side of the vegetal pole. A dense cytoplasmic band was observed at the cell surface along the deepest part of the groove and microvilli were present. Clearly defined filaments were not observed within the dense band at this stage, however.

At this stage the new unpigmented surface, exposed in the groove on the animal surface, begins to recede into the furrow. Sections transverse to the furrow near the animal pole show that a ridge, which may be 10 μm high, develops at precisely the junction between the pigmented and unpigmented cortex (Fig. 10). The plasma membrane follows the surface of the ridge, so that no part of it is extracellular. The cytoplasm in the ridge is dense (Fig. 11). Some 7-nm fibrils have been observed within it; in one instance a group of parallel fibrils was seen to extend for 3 μm within the ridge (Fig. 14).

After the completion of the first cleavage furrow, the unpigmented surface continues to recede from the exposed animal surface into the furrow and the ridges on either side of the furrow then approach each other and meet, first at each side of the egg and then progressively towards the animal pole. When the ridges have come together they develop into regions where there is a close approach of the adjacent plasma membranes to within 3 nm of each other at some points (Fig. 13). The ridges have then become thickened regions of dense cytoplasm and are seen as part of an intercellular zone of adhesion (Fig. 12) by which the daughter blastomeres are held together along their line of contact on the animal surface. These zones of contact are morphologically similar to the close junctions in early chick embryos, described by Trelstad, Hay & Revel (1967), in which the outer layers of adjacent plasma membranes are separated by a gap of 2.5–10 nm.

**DISCUSSION**

Several studies with the electron microscope on material in which cleavage takes place by constriction or furrowing have shown the presence of a layer of dense cytoplasm immediately within the plasma membrane in the furrow region. These studies include the work of Mercer & Wolpert (1958) and Weinstein & Hebert (1964) on sea-urchin eggs, Robbins & Gonatas (1964) on HeLa cells and Allenspach & Roth (1967) on chick embryonic mesenchyme, but nobody had observed oriented filaments within the layer of dense cytoplasm until the recent reports of Szollosi (1968a, b) and Schroeder (1968) both describing cleavage in coelenterate eggs. Schroeder (1968) estimated the diameter of the filaments as 3–5 nm, Szollosi (1968b) as 4–6 nm and Szollosi (1968a) as 6–8 nm, and it is uncertain whether the discrepancy between their esti-
mates and our own estimates of 8–10 nm for the corresponding filaments in newt eggs, should be taken as indicating that the filaments are different.

In *Xenopus* eggs, our preliminary observations made on sections cut transversely to the furrow at mid-cleavage also show the presence of profiles about 10 nm in diameter within a dense cytoplasmic layer at the leading edge of the furrow, where microvilli are also present.

It remains to be established whether the band of 8–10 nm parallel filaments, observed in the cortex of the newt egg at the early groove stage, was formed by a local reorganization of the randomly oriented fibrils which, before cleavage, were scattered within the dense superficial layer of the rough surface. Further work is required to characterize the surface band and its filamentous system. The present observations and those of Szollosi (1968a, b) and Schroeder (1968) are, however, consistent with a contractile band hypothesis for the formation of the cleavage groove, while the contractile band may be regarded as a modified form of the contractile ring proposed by Marsland & Landau (1954) for the case of eggs with symmetrical cleavage.

The first appearance of the filamentous cortical band across the animal surface of the newt egg coincides with the initial dipping inwards of the egg along the line of the future furrow and at the same time there is transverse wrinkling and a contraction of pigmented surface which has been recorded on ciné film. All these observations support the idea that the filaments represent a contractile system. At later stages of cleavage, however, the filamentous band persists below the leading edge of the furrow when it is below the egg surface, but then there is no similar evidence that the filamentous system continues to exert a contractile force.

Adelman, Borisy, Shelanski, Weisenberg & Taylor (1968) made a classification of cytoplasmic filaments found in a variety of cell types, and have reviewed the evidence that at least some kinds of filaments, apart from the actomyosin system in muscle, do provide a structural basis for contractility. Cloney (1966) described an oriented system of filaments 5–7 nm in diameter in caudal epidermal cells of ascidian larvae; this tissue contracts to 5% of its original length during tail resorption. In amphibian neurulae Baker & Schroeder (1967) found 4–6 nm filaments which they suggest may cause morphogenetic movements. Adelman *et al.* (1968) have proposed that filaments of 5–7 nm diameter in slime moulds are actin, and an actin-like protein has been isolated from slime moulds. In newt eggs only the fibrils observed in the dense superficial cytoplasmic layer fall in the 5–7 nm range and the parallel filaments in the furrow band are larger (8–10 nm). On the other hand, these filaments have a similar diameter to the 10-nm filaments in developing skeletal muscle cells (Ishikawa, Bischoff & Holtzer, 1968) which were found in increasing numbers after treatment with mitotic inhibitors and were shown by an immunological technique to be unlike either actin or myosin. It is noteworthy, however, that Sakai (1968) has demonstrated the presence of contractile proteins in the cortices of cleaving sea-urchin eggs.

Selman & Waddington (1955) suggested that the dipping inwards of the early groove in newt cleavage, the surface movements and the transverse wrinkling were due to contraction in a sheet of subcortical gel formed in the plane of the future furrow. It is now more probable that the contractility is in the dense fibrillar band of the cortex.
Electron microscopy cannot disprove the existence of a sheet of cytoplasm in the gel state, but the former hypothesis was supported by observations of modified cytoplasm, including displaced pigment granules and yolk platelets, in the plane of cleavage ahead of the furrow. These observations were made by light microscopy with paraffin sections and some of them need to be reassessed since it was not realized that most of the mid-cleavage furrow in *T. alpestris* is so narrow (Fig. 8) as to be below the resolution limit of the light microscope. Similarly in the light micrographs of Zotin (1964), the structures labelled as showing late diastema in axolotl eggs may represent fully-formed furrow with the opposite surfaces in contact. On the other hand, the pictures of early diastema (plate 1, fig. D, of Zotin, 1964; plate 2, fig. 6, of Selman & Waddington, 1955) have probably not been misinterpreted and the diastema may play a role in determination of the cleavage plane as Zotin (1964) suggested. Moreover, Kubota (1969) working with frog eggs was able to displace experimentally the subcortical cytoplasm ahead of the furrow and showed that in its subsequent progress the furrow deviated from its normal course to pass through the new position of the displaced cytoplasm. In the present work no structural modifications were observed below the leading surface of the furrow apart from an accumulation of granules.

The present observations support the conclusions of Schechtman (1937), Selman & Waddington (1955) and Zotin (1964) that in cleavage of amphibian eggs the new cell surface is formed only in the furrow region. In the newt egg, the new cell surface is distinguished from the old not only in having less pigment, but in being much smoother and with less material outside the plasma membrane. Moreover, the join between the new and old surface is clear and abrupt. Buck & Krishan (1964) considered that in epithelial cells of frog tadpoles the original cell surface did not expand into the cleavage furrow since it was held to adjacent cells by numerous desmosomes. Buck & Tisdale (1962) also concluded that cleavage in sarcoma cells of rat was accompanied by local synthesis of new plasma membrane in the furrow region.

The present work describes the growth of new unpigmented cell surface in a furrow which extends progressively downwards through the egg from the animal to the vegetal surface during cleavage. Having regard to theories of cleavage based on an expansion or stretching of the original surface (see Swann & Mitchison, 1958; Wolpert, 1960), some consideration has also been given to the idea that the unpigmented surface of the furrow might represent an expansion of pigmented surface in which the pigment is diluted by the expansion. In the present work a strip of white surface 80 μm wide was observed across the animal surface at the bottom of the groove soon after it had formed, so that an upper limit of 40 μm can be put upon the initial width of the strip of pigmented surface which can be supposed to expand. From this it may be calculated that an increase of at least 80 times the initial area must take place to provide all the unpigmented surface. So great an increase is not possible unless many times the initial numbers of lipid and protein molecules are assembled in the new cell surface during the process. Hence the idea of active growth of new surface in the furrow of the egg during cleavage is supported.

Wolpert (1963) estimated that surface foldings and convolutions in sea-urchin eggs could account for a 30% increase in surface during cleavage if they became flattened.
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In newt, the extent of surface irregularities observed before cleavage remains unaltered throughout cleavage except in a narrow strip less than 20 μm wide in which irregularities do become flattened at the early groove stage. It has been estimated that this flattening could provide less than 1% of the new surface formed during cleavage in the newt egg.

Further work is needed to establish whether the furrow grows only at its leading edge or over a considerable area behind the leading edge. Moreover, it remains to be established whether furrow growth takes place accompanied by synthesis of protein and lipid material in the cytoplasm adjacent to the furrow or whether furrow growth takes place merely by the proper assembly of macromolecules synthesized before cleavage.

The occurrence of microvilli at the leading edge of the amphibian cleavage furrow may indicate where growth of new cell surface takes place. Microvilli may represent a site of local synthesis of surface in excess of that which can, at the particular time and place, be integrated into a general expansion. Microvilli should not be confused with the dissimilar surface blebs which Robbins & Gonatas (1964) observed on the polar surface of cleaving HeLa cells. The electron micrographs of these authors also showed microvilli in the furrow of a late telophase cell; Schroeder (1968) reported microvilli in the furrow of *Somatica* eggs and Jurand & Selman (1969) found microvilli in the fission furrow of *Paramecium* where there was increase of surface.

In addition to studies on cytokinesis in plants (e.g. Whaley, Dauwalder & Kephart, 1966; Hepler & Jackson, 1968), several ultrastructural studies of cleavage in certain animal tissues have provided evidence that the new cell surface may form by the coalescence of vesicles which line up along the course of the future furrow (Odor & Renninger, 1960; Humphreys, 1964; Murray, Murray & Pizzo, 1965; Thomas, 1968). The present observations have not established a role in newt cleavage for the many vesicles, 0.1–0.2 μm in diameter, which were observed in the pigmented cortex and near the growing furrow where they may have been transported by streaming. Vesicles were rarely observed in cytoplasm ahead of the furrow and after a careful search only 2 instances were found of vesicles apparently fusing with the surface of the furrow.

The present ultrastructural studies on newt cleavage provide support both for a contractile band hypothesis to account for the initial dipping inwards of the groove and also for the idea of the growth of new cell surface during the extension of the furrow (cf. Schechtman, 1937). There are many theories of cleavage (reviewed by Wolpert, 1960), most of which are based upon single simple ideas. However, the cleavage process in any kind of cell is probably more complex than seems to have been generally recognized, and it is unlikely that a single hypothesis can explain all the observations. Thus in the cleavage of the sea-urchin egg it has been concluded that the furrow is initially formed by stretching the existing surface (Wolpert, 1960; Hiramoto, 1968) but this conclusion does not indicate the time and place of synthesis of new cell surface during the cleavage cycle. However, Wolpert (1963) points out that observations on the surface movements of echinoderm eggs (for examples, see Motomura, 1940, 1950; Dan & Ono, 1954; Wolpert, 1966) suggest that, in this case, new cell surface forms in the plane of cleavage following furrow formation. On this basis the mechanism of cleavage in the newt egg and the sea urchin now appear to be more similar.
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Fig. 2. Onset of cleavage. Transverse section through the shallow groove at the animal pole. The characteristic rough surface of the egg is interrupted by stretches of smoother surface (arrowed). There is a band of dense peripheral cytoplasm, \(0.1 \mu m\) thick, below the smoother regions. A cortical layer, about \(5 \mu m\) thick, contains vesicles (v), pigment granules (p) and mitochondria. The internal cytoplasm of the egg includes yolk platelets (y) and lipid droplets (l). \(\times 6000\).

Fig. 3. Early to mid-cleavage. Surface layers of the egg lateral to the groove. The irregular rough surface is covered by a layer of extracellular material about 50 nm thick. Within the plasma membrane there is a layer of dense granular cytoplasm in which there are fine fibrils (f) and glycogen granules (g). Below this lies the pigmented cortex in which there are many vesicles (v), \(0.1-0.2 \mu m\) in diameter. \(\times 50000\).

Fig. 4. Early to mid-cleavage. Surface layers of the egg from the unpigmented sides of the groove (also see Fig. 1 c). The surface contours are smooth. There is a comparative absence of extracellular material and the layer of dense peripheral cytoplasm is thinner (compare Fig. 3). The vesicles in the cortical layer are less numerous than below the rough surface. g, glycogen granules. \(\times 50000\).
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Fig. 5. Early to mid-cleavage. Transverse section through the bottom of the groove. Microvilli containing fibrillar material (f) project from the surface. Below the surface there is a band of parallel filaments (fil) seen here in cross-section. There appear to be fewer filaments below the bases of the microvilli. × 45000.

Fig. 6. Early to mid-cleavage. Transverse section through the bottom of the groove. Higher magnification of the superficial layers to show, in section, the band of filaments (fil), 8–10 nm in diameter. × 100000.

Fig. 7. Early to mid-cleavage. A section in the plane of the prospective furrow shows the filaments in longitudinal view. fil, filaments. × 100000.
Fig. 8. Mid-cleavage (as in Fig. 1 d). Sectioned at right angles to the growing furrow. Above the bulbous leading edge of the furrow, the adjacent plasma membranes are closely apposed except at a few isolated points (arrowed) where there are gaps between the membranes. There are pigment granules around the leading edge of the furrow. $\times 3000$.

Fig. 9. Mid-cleavage. The marginal cytoplasm round the bulbous end of the furrow contains obliquely sectioned filaments of 8–10 nm diameter. Microvilli project into the cavity formed by the furrow. fil, filaments. $\times 40000$. 
Cleavage in the newt's egg
Fig. 10. Late cleavage. Light micrograph of a section through the animal surface of the egg. The boundary between the rough pigmented surface (r) and the smooth unpigmented surface (s) is clearly marked by a protruding ridge (compare Fig. 11). x1500.

Fig. 11. Late cleavage. The ridge is seen to be a protrusion of the egg cytoplasm. The plasma membrane (visible at higher magnifications) follows the contours of the ridge. Within it there is a loose meshwork of granular cytoplasm with glycogen granules (dark dots) and a few vesicles. Near the middle of the ridge there is a strand of denser cytoplasm, like that seen at higher magnification in Fig. 14. x10000.

Fig. 12. Post-cleavage. The junction between the 2 daughter blastomeres at the animal pole. The external surfaces of the blastomeres retain the characteristics of the rough surface of the uncleaved egg, whereas the internal surfaces are similar to the new smooth surface although some pigment extends a short distance into the egg where the cells meet. Apposed plasma membranes between the dense outer zones of cytoplasm form a close intercellular junction (j). At a deeper level the gap between the cells varies in width. x12500.

Fig. 13. Post-cleavage. Higher magnification of the close junction at the external surface. Note the close approach of the plasma membranes of the daughter blastomeres (arrowed). x120000.

Fig. 14. Late cleavage. Detail of a dense cytoplasmic strand in the ridge. It is composed of a bundle of fibrils each about 7 nm in diameter. x50000.
Cleavage in the newt's egg

10

11

12

13

14

r

s

j

10 μm

1 μm

0.1 μm

0.1 μm