Cleavage in the chick embryo

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SUMMARY

Chick embryos ranging from the stage of first cleavage to that of about 700 cells were removed from the oviduct and examined by transmission electron microscopy. Beneath the cell membrane is a yolk-free cortical region containing microfilaments. Beneath this lies cytoplasm which contains yolk spheres which are graded in size, the dorsal ones being smaller than the ventral ones. The subgerminal periblast possesses a greater proportion of yolk to cytoplasm than do the cells proper, but it merges with the cytoplasm at the incomplete borders of the ‘open’ cells.

Specialized accumulations of membranes lie in the marginal periblast, and it is suggested that they play a role in cell membrane formation.

(This paper is dedicated to Professor Silvio Ranzi on the occasion of his 75th birthday)

INTRODUCTION

The cytological events occurring during mitosis are similar in most types of vertebrate cell, though they vary in detail. The most usual example of cell division, however, is probably that found during cleavage in large-yolked species such as the fowl. According to the classical descriptions, which are based on the study of paraffin sections by light microscopy (Patterson, 1910; Olsen, 1942), a number of ‘open cells’ develop. An ‘open cell’ is one which is bounded by cell membrane on only a part of its surface, and whose cell contents are in open communication with the yolk (Fig. 1). It is supposed that as each ‘open cell’ completes mitosis, one daughter nucleus remains in situ and the other migrates out together with some cytoplasm into the adjacent yolk, forming a region known as the periblast, which is part yolk, part cytoplasm. Thus the periblast lies around the periphery of the embryo (marginal periblast) and beneath it (subgerminal periblast). The cell membrane now extends down between the two cell nuclei, so that one of the cells loses its contact with the periblast and hence with the yolk, and is no longer ‘open’. At the same time, a new ‘open cell’ has formed around the other daughter nucleus.

Hitherto, the only detailed modern study of cleavage in the chick embryo

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Fig. 1. Diagram to illustrate the structure of the open cells in early cleavage. (A) Four stages seen from the dorsal side of the embryo. The yolk is not illustrated but the periblast is visible (from life). (B) Transverse section through the edge of an early embryo.
was that of Gipson (1974), although she restricted herself to a description of the cleavage furrows during the first three divisions. In this paper we have not given space to those aspects of our results which are a mere confirmation of hers. Instead, we have investigated the structure of the ‘open cells’ by light and electron microscopy. In view of the importance of ribosome formation in young embryos (see Discussion) we have also attempted to find out whether nucleolus are present in the early stages and, in addition, we have considered the nature of the cleavage furrows and the possible mechanism of their formation.

**MATERIALS AND METHODS**

Thirty-five laying hens were killed, usually by intravenous injection of sodium barbiturate, but occasionally by ether anaesthesia. The cleaving embryo was dissected from the oviduct and immediately fixed at room temperature (15–20 °C) and at pH 7.0–7.2 with either diluted Karnovsky's fixative (Karnovsky, 1965) or with Burnside's fixative (Burnside, 1971). In some specimens, the entire yolk was plunged into the reagent and the young embryo dissected from it. In others, the embryo was removed from most of the yolk before fixation. After remaining in the fixative for periods varying from 1 h to several days, the embryo with adherent yolk was washed in cacodylate buffer (pH 7.0–7.2) and treated with buffered 1% osmium tetroxide for 1 h at pH 7.0. During dehydration with ethanol, the specimens were stained with Araldite or Epon. Sections were stained with lead citrate and examined with either a Siemens Elmiskop 1 or with an A.E.I.-801 electron microscope.

Thick sections were routinely obtained from the same blocks and were stained with a 1% aqueous solution of toluidine blue and examined by light microscopy. Twenty-three additional specimens were embedded in soft plastic and serially sectioned at 4 μm. They were stained with acid fuchsin and toluidine blue according to the technique of Ruddell (1971).

Three further specimens were treated at 37 °C with a 1% solution of α-amylase made up in 0.85% saline at pH 6.0. Each specimen was cut into three pieces, one being treated for 1 h, another for 3 h, and the third for 6 h. Control specimens were incubated with saline for corresponding periods. After treatment, specimens were washed briefly in fresh saline and processed in the same way as the main group of embryos.

In all, 82 specimens were examined ranging in development from the stage of first cleavage to about 700 cells. In the earliest stages when all the cells were still open, it was sometimes difficult to decide how many were present, especially since the furrows soon became irregular. The following criteria were therefore adopted:

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<th>No. of cleavage furrows</th>
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RESULTS

The general relationship of the cleaving embryo to the vitelline membrane is shown in Figs. 2 and 3. A fine granular material lies between the vitelline membrane and the embryo, and extends into the furrows between the cells. The structure of the vitelline membrane of the fowl has been described elsewhere (Bellairs, Harkness & Harkness, 1963; Jensen, 1969) and will not be discussed here.

Until about the 16-celled stage, all cleavage cells are 'open' (Romanoff, 1960) and in direct communication with the periblast (Figs. 1, 2). When these 'open' cells are examined by electron microscopy three major zones are apparent: the cell membrane, the cortical region and the yolky cytoplasm (Fig. 3). The last grades into the periblast which is rich in yolk spheres and poor in cytoplasm, and this in turn grades into the totally non-cytoplasmic yolky region (see Fig. 1). By the 16-celled stage the most centrally situated cells have become completely surrounded by cell membranes, though those in the more peripheral regions remain 'open'. Eventually, the 'open' cells disappear.

Gipson (1974), who examined only 2-, 4- and 8-cell stages, classified the furrows as being V-shaped, U-shaped or pendulum-shaped, in longitudinal section. We have not found it possible to distinguish between the V-shaped and the U-shaped since many of the variations which occur may be the result of different angles of sectioning. We prefer to rename both of these types as shallow furrows (Figs. 4, 5). The deeper, pendulum-shaped furrows are characterized in section by a narrow neck which swells into a bulbous region at its deepest extent. We have also seen an additional type of furrow not described by Gibson, which is characterized by relatively smooth walls and apparently by tight junctions (Fig. 3). We have named this slit-like. This too is found in later stages and is probably restricted to the most peripheral regions; it does not seem to be related to the stage of the embryo. We have seen all three types of furrow at all stages examined from the 2-cell to the 700-cell stages. Many of the furrows, especially the pendulum-shaped ones, possess highly elaborate...
Cleavage in the chick embryo
Fig. 4. Diagram to illustrate the three types of cleavage furrow. (A) Slit-like; (B) shallow; (C) pendulum-shaped.
structures at the base (Figs. 6, 11). Typically, each consists of a furrow-base-body with branching processes extending from it. (For a full description, see Gipson, 1974.) Examination of many thick sections by light microscopy has convinced us that there is only one region of base-body per furrow, though our electron micrographs have demonstrated that in that location the furrow-base-body may have several attachments (Fig. 6).
By the 64-cell stage the open cells are restricted to the peripheral region. The cell membrane of each open cell is continuous with the cellular membrane covering the periblast. Fig. 7 is a micrograph of a section at the angle between an open cell and periblast and shows a region of projections resembling a furrow-base-body. Similar projections are shown in Fig. 8 on the surface of fully formed cells from late cleavage (about 700 cells).

Figures 11–13

Fig. 11. Electron micrograph of a section to show the root region (arrowed) which lies ventral to the furrow-base-body. Taken from an embryo of 16 cells. × 6250.

Fig. 12. Electron micrograph of a section in the region where the periblast merges into the yolk. Note the large closely packed yolk granules, each with a densely stained core (c) and a less dense peripheral region (p); note also the mitochondria (m). × 6250.

Fig. 13. Electron micrograph of a section through the nucleus (n) in an embryo of 64 cells. Note the absence of nucleoli. × 5000.
Cleavage in the chick embryo
The cell membrane along the dorsal surface of the open cells exhibits many pinocytotic pits and vesicles and is also extended out as microvilli. Beneath the cell membrane lies a cortical zone. This is free of yolk and usually about $\frac{1}{2} \mu m$ thick. It contains an array of microfilaments which appear to run parallel to the surface of the membrane, though it is not always easy to see them in the older cleavage stages. This suggests that they are frequently not arranged to run in a predominantly single direction. In the microvilli (Fig. 10), however, they tend to lie in conspicuous parallel bundles and are sometimes interspersed with fine granules which individually measure about 10 nm across and appear to be ribosomes. Occasionally glycogen granules are present in the cortex.

Beneath the cortex is the cytoplasmic region which contains the same components in both open and closed cells, both in early and later cleavage. It is characterized by the presence of densely staining granules each about 300 nm in diameter. These granules are often arranged in clusters and are considered to be glycogen. In specimens treated with $\alpha$-amylase many of the granules sometimes extended into the projections in the furrows.

Other components of the cytoplasm include yolk spheres (Fig. 11), mitochondria, ribosomes, Golgi bodies and annulate lamellae. Granular endoplasmic reticulum is scarce but smooth surfaced vesicles are common.

Two regions of highly specialized cytoplasm may be noted. The membranous vesicular reticulum (Fig. 9) tends to lie near the dorsal surface, especially in the marginal periblast of young cleavage stages. It consists of a dense accumulation of small vesicles which individually measure between about 0.2 and 0.5 $\mu m$ in diameter and which frequently contain a fine granular material. Larger vesicles are also present, though these tend to be relatively empty.

The second type of specialization is seen directly beneath many of the furrow-base-bodies (Fig. 11). It consists of a dense concentration of cytoplasm which passes like a root deep into the periblast and consists of an aggregation of Golgi bodies, vesicles and mitochondria (Fig. 4C).

The yolk spheres present in the cytoplasm are usually of the relatively simple type described by Bellairs (1958) in which each consists of a densely staining granular core surrounded by a less dense zone (Fig. 11). Some of those lying most dorsally measure as much as 10 $\mu m$ in diameter and are separated by cytoplasm. More deeply situated yolk spheres are larger with less cytoplasm between them. This changing relationship between the size of the yolk spheres and the amount of cytoplasm is especially noticeable in the open cells where the morphological size gradient changes in a proximo-distal as well as a dorso-lateral manner, until in the region of undoubted periblast (Fig. 12) very few cytoplasmic structures can be seen among the yolk spheres. Lipid drops of the type seen in older chick embryos (see Bellairs, 1958) are seldom visible in these early stages. Similar observations on the intracellular yolk spheres have been made by Emanuelsson & Von Mecklenburg (1968).

A remarkable feature of the investigation is our inability to identify nuclei in
Cleavage in the chick embryo

the early cleavage stages or in the periblast, despite the fact that we have searched through very many paraffin and plastic sections by light microscopy, as well as thin sections by electron microscopy. Furthermore, we have seen mitotic figures only rarely in open cells (Fig. 2). We have, however, frequently seen nuclei in closed cells (Fig. 13). These nuclei do not appear to possess nucleoli but are sparsely packed with granules.

DISCUSSION

Many theories have been proposed to explain the mechanism of cleavage in young embryos (reviewed by Wolpert, 1960). Most of these are based on the study of echinoderm eggs which possess little yolk and so undergo total (holoblastic) cleavage. Large yolked eggs cleave in a different way (meroblastic cleavage), forming ‘open’ cells at least in the early stages. The mechanism of cell division in large yolked species must therefore differ in some respects from that in echinoderm eggs, and theories based on echinoderm cleavage cannot be applied to the chick without some modification. In general, most theories include the following features: first, that the plane of cleavage is related to the mitotic apparatus of the cell, but cleavage may nevertheless be initiated in the absence of the mitotic figure. There is experimental evidence that in many echinoderms the asters play an essential role (Rappaport, 1973), but we have no evidence as to the part played by mitotic spindles or asters in the chick.

Second, there is a growth of new cell membrane, and there are many theories as to how this is brought about (see Singer & Rothfield, 1973). One theory is that cytoplasmic vesicles fuse with the existing cell membrane (Arnold, 1968, 1969). The presence of many cytoplasmic vesicles in the chick suggests that this might be the mechanism, though other factors may be involved. For example, in *Xenopus* there is an insertion of precursors at intervals along the existing membrane (Bluemink & DeLaat, 1973) and it is possible that the same process occurs in the chick.

A third feature of all cleaving embryos is the presence of microfilaments running parallel to the surface membrane. These structures have been described in many species, and by finding them in the chick, we confirm the observations of Gipson (1974). They are also present in the embryos of cephalopods (Arnold 1968, 1969, 1971), which resemble the chick in undergoing meroblastic cleavage.

It has often been suggested that each furrow is produced by contraction of the microfilaments (e.g. Arnold, 1968, 1971; Szollosi, 1970; Bluemink, 1973; Perry, 1975) and there is some evidence that if the microfilaments are cut (Arnold, 1971) furrow formation is impaired. The process may also be upset by treating the embryo with cytochalasin B (Arnold & Williams-Arnold, 1974; Bluemink & DeLaat, 1973), though the effect on the microfilaments may then be an indirect one (Bluemink, 1971). It is generally considered that contraction of the microfilaments leads to the folding and wrinkling of the cell membrane
which is so characteristic a feature not only of cleaving eggs (e.g. Bluemink, 1971; Monroy & Baccetti, 1975; Arnold, 1969) but also of many cells in mitosis (e.g. Schroeder, 1968; Scott & Daniel, 1970). It may be noted, however, that many of the projections, especially those in the necks of the pendulum-shaped furrows, possess many more clearly arranged bands of microfilaments than do the walls of the furrows, and this suggests that the projections are actively motile structures rather than merely folds pulled passively into this shape. It seems possible that they play a role in preventing the two sides of the furrow becoming pulled widely apart. The tight junctions in the walls of the furrow probably also aid in keeping the developing cells in contact. Although gap junctions are present shortly after incubation of the laid egg begins (Bellairs Breathrach & Gross, 1975), they do not appear to be present in the early cleavage stages. There is extensive evidence in the literature that electrical coupling of cells takes place via the gap junctions (Gilula, 1974) but it seems unlikely that specialized contacts would be required for cell communication when body cells are still present.

The furrow-base-body appears to be associated with the production or accumulation of new membrane. Gipson (1974) has suggested that it is analogous to the midbody of cleaving holoblastic eggs. This seems unlikely, since the furrow-base-body does not resemble the midbody found in the chick embryo at later stages (Bellairs & Bancroft, 1975). Furthermore, a midbody proper is formed at the point of constriction of two daughter cells, but there is no comparable constriction in the cleavage stages of open cells. We suggest that the furrow-base-body is related to the burrowing of the furrow into the periblast, and may be associated with the change of direction which occurs when the furrow begins to extend laterally to form the ventral border of the cells. Arnold (1969) has described a specialized region at the base of the cleavage furrows in the cephalopod, *Loligo*, which consists of a network of tubules, though he was unable to ascribe a function to it. Both the cephalopod tubular network and the avian furrow-base-body occupy comparable positions in meroblastic eggs; each is a highly membranous structure. Nothing is known about the role of the root region beneath the furrow-base-body, nor about the processes which prevent the furrow from passing deeper and deeper toward the yolk. It seems likely, however, that a furrow reaches its maximum depth when the proportion of yolk to cytoplasm passes a certain threshold. A similar situation appears to hold in *Loligo* (Arnold, 1971) though there the yolk and the cytoplasm are sharply separated from one another.

The nature of the periblast and of the open cells in the chick has received little attention in recent years. The term periblast is used to cover the regions which lie both ventral and peripheral to the closed cells. The cytoplasmic components of the ventral periblast appear to be similar to those of the embryo proper, though the relative proportions of yolk and cytoplasm gradually change between the cells and the periblast and the yolk. In particular, small
patches of glycogen granules and mitochondria are visible in the regions which would otherwise be considered to be entirely non-cytoplasmic. The specialized membranous structures illustrated in Fig. 9, however, have been seen only in the new membrane (as discussed above). Significantly, we have never been able to identify nuclei in either of the periblast regions whether by light or by electron microscopy. Patterson (1910) also failed to find nuclei in the periblast. Bekhtina (1960) reported on the basis of a light microscopic study that the cleavage nuclei of the chick were found at the depths of the furrows. We have not seen nuclei in these regions and in our opinion, Bekhtina mistook the pendulum at the base of the furrow for a nucleus. Nuclei are notoriously difficult to distinguish in light microscope sections of yolky material.

Similarly, we have failed to find nuclei at all in the open cells of the early stages, though we have on one occasion found a mitotic spindle in an open cell of a 12-cell embryo. Gipson (1974), though not discussing the problem, did not illustrate or mention the nuclei, whilst Emanuelsson (1965) reported a failure to find mitotic figures in embryos with less than 30 cells. Negative results being notoriously unsatisfactory, the situation needs further analysis, but we would like to emphasize that if it were established that nuclei were not present in all the open cells or in the periblast, then traditional ideas on the mechanism of expansion of the early cleavage embryo would need revision. It has sometimes been suggested that amitosis can occur during cleavage in birds (Patterson, 1910; Emanuelsson, 1965), but we would be reluctant to accept this idea without firmer evidence.

One possibility is that the cytoplasm of the early embryo begins to undergo division as a result of activation by the many accessory sperm nuclei still present at this stage, and that the open cells only later become colonized by embryonic nuclei. Certainly, natural parthenogenesis in birds is now well established (Olsen, 1966; Lorenz, 1975) and this implies a ready ability to divide without a full chromosomal complement.

By contrast, nuclei are easily seen at later stages of cleavage when closed cells are present (Fig. 13). At first they appear to lack nucleoli and these have not been seen by us until about mid-cleavage. This supports the findings of Wylie (1972) that RNA synthesis does not begin to take place until cleavage is well advanced.

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REFERENCES


Cleavage in the chick embryo


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