AN ELECTRON MICROSCOPIC STUDY OF THE DEVELOPMENT OF THE CLEAVAGE FURROW IN MAMMALIAN CELLS

ROBERT C. BUCK, M.D., and JAMES M. TISDALE

From the Department of Microscopic Anatomy, University of Western Ontario, London, Canada

ABSTRACT

The process of cytoplasmic cleavage has been studied in thin sections of rat erythroblasts and the cells of mouse leukemia and Walker 256 carcinoma of the rat. The development of the cleavage furrow begins in relation to the mid-body, which, earlier, appears on the equatorial plane in association with the continuous fibers of the spindle. The earliest evidence of a cleavage furrow is the presence of a vesicle or vesicles close to the mid-body. Subsequently, many smaller vesicles are seen in the equatorial plane. The cleavage furrow probably develops by the fusion of these vesicles so that a new plasma membrane is formed between the daughter cells, and extends from the telophase intercellular bridge to the cell margin. During the stage of formation of the vesicles, cisternae, believed to be part of the endoplasmic reticulum, assume an intimate relationship with the cleavage plane, and they may perhaps be involved in the formation of the vesicles.

INTRODUCTION

Cytokinesis has been studied by many techniques in many kinds of cells, plant, animal, and protist. Numerous theories have been proposed in attempts to explain the process, and two reviews of these have recently been published (13, 15). At the present time no single theory of cleavage appears to be applicable to all types of cells. However, Mazia (7) has recently pointed out that a mechanism of cleavage common to a variety of types of cells might yet be discovered through the use of newer techniques. He has suggested that the process of intracellular synthesis of new plasma membrane at the cleavage furrow, reported earlier in plant cells (2, 11), might apply also to other kinds of cells. This mechanism of cleavage consists in the development, in the equatorial plane, of vesicles which then fuse together so that a continuous membrane of each daughter cell is produced. Evidence exists for this mechanism of cleavage in fish eggs (14), *Thalassema* eggs (14), sea urchin eggs (9), and amphibian eggs (12), and a somewhat similar process has been observed in rat embryonic liver cells (3).

In the present report the development of the cleavage furrow is studied in relation to the spindle and the mid-body in mammalian cells. The fine structure of the mid-body has been described earlier (1).

METHODS

Observations have been made on mitotic cells of bone marrow (erythroblasts), prepared as described previously (1), and on cells of the Walker 256 carcinoma and two leukemias developing spontaneously in old Swiss mice. The tumor tissue was prepared for electron microscopic examination in the same manner as the bone marrow.

OBSERVATIONS

Most of the changes in cellular morphology to be described have been seen in each of the three

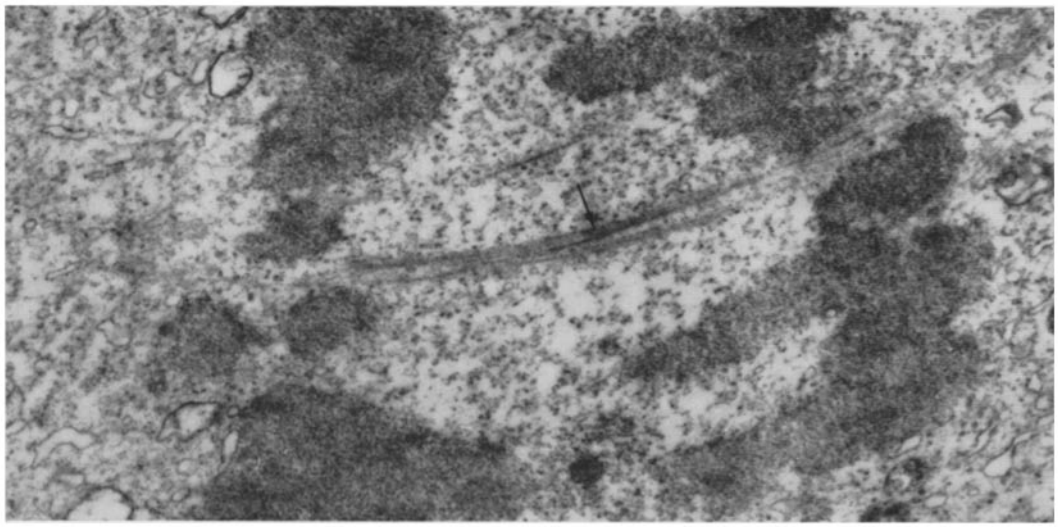

FIGURE 1

Anaphase in mouse leukemia cell. Fibrillary substance extends, as the continuous fibers of the spindle, between the separating chromosome sets, and continues toward the poles of the cell by passing between the individual chromosomes. At the plane of the equator a short length of fiber shows a pronounced increase in density (arrow). × 14,000.

FIGURE 2

Telophase in Walker 256 carcinoma. The nuclear membranes have largely re-formed and the daughter nuclei are dense, disc-like structures. On the equatorial plane two regions of the spindle show a pronounced increase in density (arrows). × 15,000.

types of cells studied. We believe that there is probably not a fundamental difference between them in this respect. On the contrary, the differences between the cells, when comparable stages of mitosis are examined, seem to stem mainly from the relative size of the cells, size of nuclei, degree of prominence of the cisternae of the endoplasmic reticulum (ER), and other quantitative features.

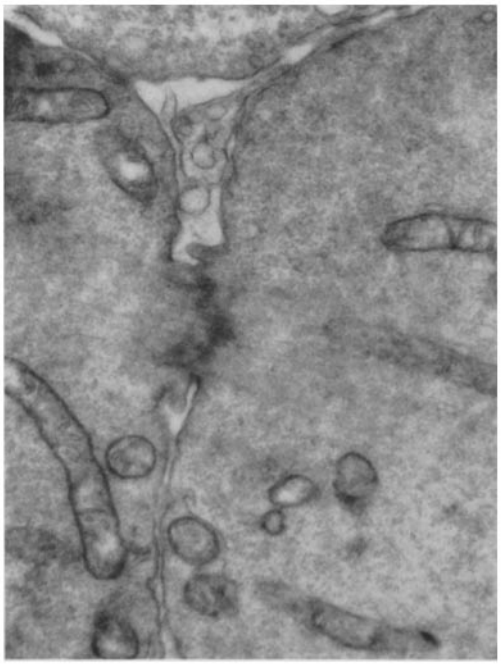

FIGURE 3

Mid-body in erythroblast after development of the cleavage furrow. It consists of a dense plate of fibrillary material, apparently developed by the aggregation of densities on the spindle of the type shown in Figs. 1 and 2. × 23,000.

Cytokinesis extends through a considerable part of the period of mitosis. The anaphase flattening of the cell may be considered the beginning, and the end is reached only when the telophase bridge connecting the daughter cells finally ruptures.

In early anaphase, as the cell begins to lengthen, the continuous fibers of the spindle already show points of increased density (Fig. 1) which later form the mid-body (1). With the onset of telophase and the beginning of the application of the nuclear membrane to the chromosome masses, the cell shape changes slightly as the equatorial region undergoes some narrowing. At this time the short lengths of density on the fibers of the spindle have become condensed in the axial part of the equatorial plane (Fig. 2).

By the time the nuclear membrane has developed to enclose the chromosome mass on the equatorial as well as on the polar surface, the densities on the spindle have formed a fibrillary plate which constitutes the mid-body (Fig. 3).

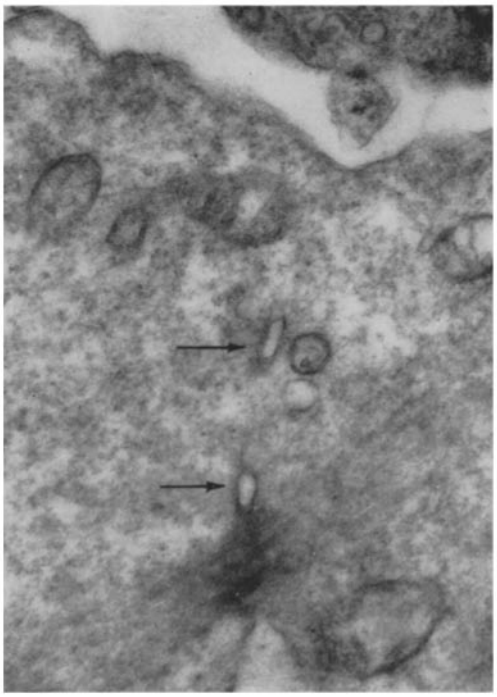

FIGURE 4

Erythroblast mid-body at an earlier stage than that seen in Fig. 3, showing its eccentric position and the presence of an oval profile of a vesicle in the cytoplasm close to it (lower arrow). A second oval profile is also present on the equatorial plane (upper arrow). × 34,000.

The mid-body lies in the equatorial plane and, in the erythroblast, over to one side of the long axis of the cell. Therefore the cleavage furrow has to develop farther on one side of the mid-body than on the diametrically opposite side. In some sections, depending upon the plane of sectioning, very little cytoplasm may separate it from the plasma membrane at one side of the cell. At this time there are observed some profiles of vesicular structures in relation to the mid-body (Fig. 4).

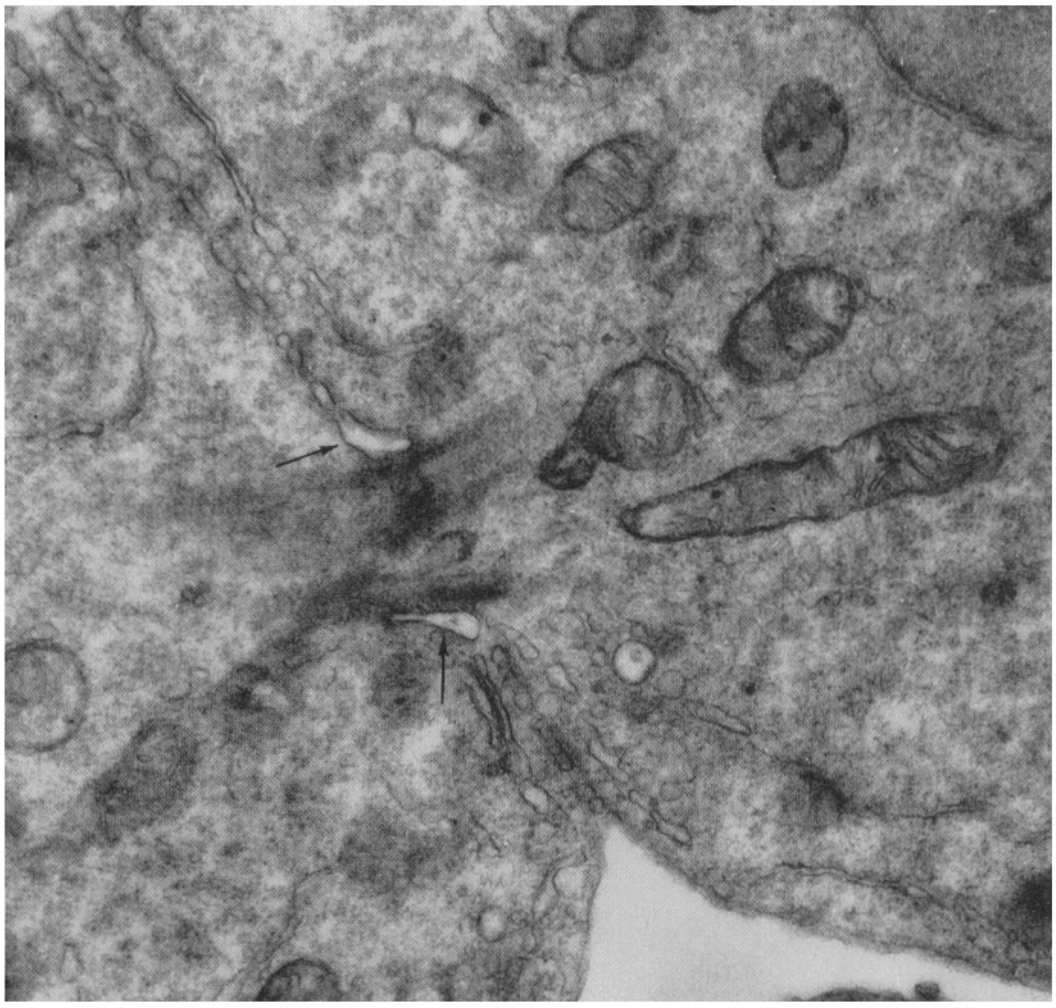

FIGURE 5

Cleavage region of an erythroblast at the stage of formation of multiple vesicles on the equatorial plane. The midbody consists of dense, fibrillary material in the bridge joining the daughter cells. On each side of it is the elongated profile of a vesicle (arrows) which is larger than the other vesicles. The latter extend from the larger vesicle as a straight row to the cell margin. On each side of the equatorial plane is a much perforated, double-walled cisterna, the profile of which is reflected away from the cleavage plane as it approaches the spindle. A part of the nucleus of one daughter cell is seen in the upper right corner. × 36,000.

Usually, a membrane-bounded vesicle, the contents showing almost no density, is seen against the mid-body on the side facing the greater thickness of cytoplasm. In other sections the profile of a vesicle is seen at either side of the mid-body. Serial sections show that these profiles may fuse into one. In other words, there appears, in some cases at least, to be a ring-shaped vesicle encircling the mid-body. Such a structure cannot be illustrated adequately in a single micrograph, and our interpretation has been built up by examining several cells at this stage, some of which have been cut serially. It must be admitted, however, that we have been able to find only a very small propor-

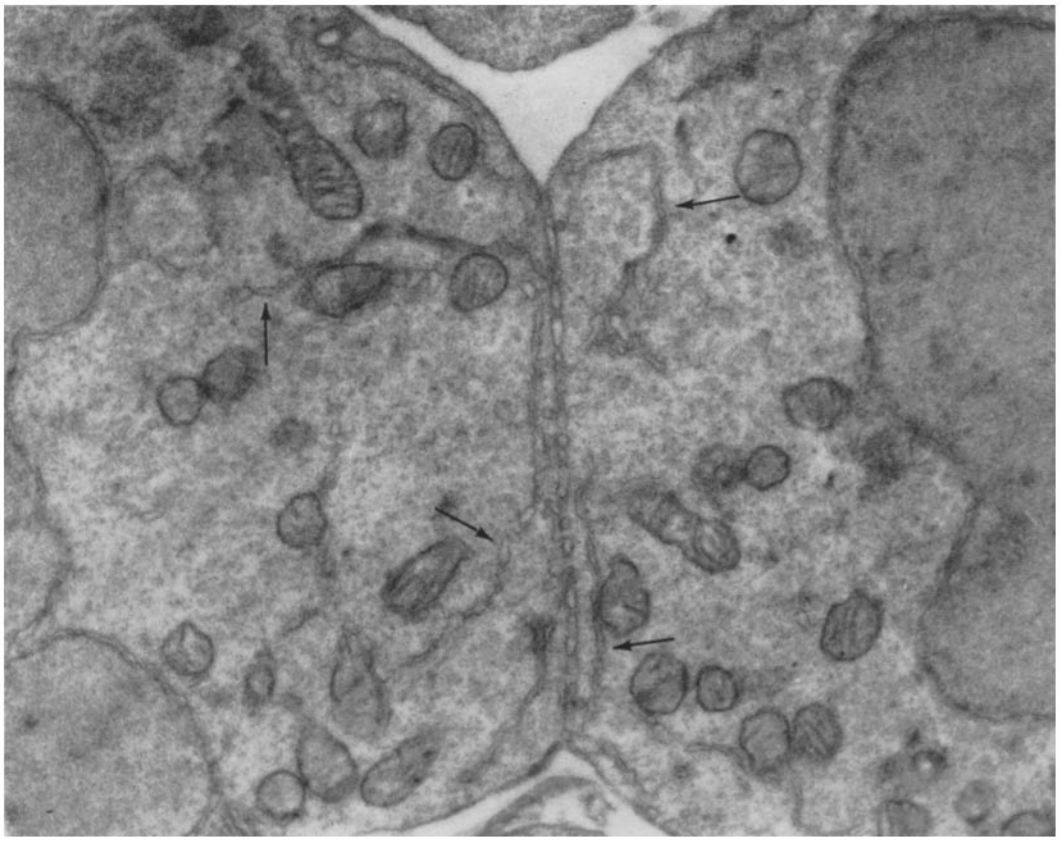

FIGURE 6

The same cell as that shown in Fig. 5, sectioned at a level that does not include the spindle. This micrograph shows the extent of the cisternae (arrows) and their disposition in relation to the cleavage plane, plasma membrane, and nuclei. If the distribution of the cisternal profiles seen here is considered in relation to that shown in Fig. 5, it is apparent that the cisterna of each daughter cell is in the form of a large, incomplete ring which encircles the spindle. This section passes to one side of the center of the ring. × 18,000.

tion of mitotic cells in this stage of development. We conclude that this phase occupies a small part of the time of the mitotic cycle.

Images have been obtained showing what are interpreted as an immediately succeeding stage, in which a row of vesicles lies on the equatorial plane. The cell illustrated in Figs. 5 and 6 has been intensively studied by means of twelve sections through it, and it appears to us quite instructive as to the events at this moment in time. The mid-body is recognized by the presence of fibrillary material of high density. The ring-shaped vesicle is considerably larger than the other vesicles lying in the equatorial plane. The latter consist of roughly spherical, membrane-bounded structures measuring from 40 to 70 mμ in diameter. Since about thirty of them are seen in one section, several hundred would probably be present in the whole equatorial plane.

On each side of the row of vesicles in Figs. 5 and 6 is seen the profile of an incomplte double-membrane system or cisterna. The disposition of these cisternae has been studied in only a few cells, but it is hard to avoid the conclusion that they are, in some way, associated with the development of the vesicles of the equatorial plane. From the series of sections through these daughter cells it is obvious that the cisternae are symmetrically

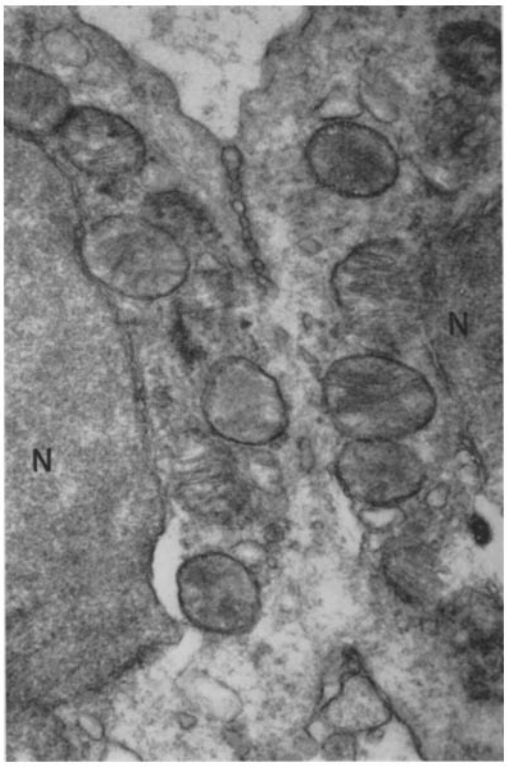

FIGURE 7

Mouse leukemia cell in telophase, showing a short row of vesicles on the equatorial plane. The section does not pass through the spindle. Nuclei of daughter cells are shown (*N*). × 29,000.

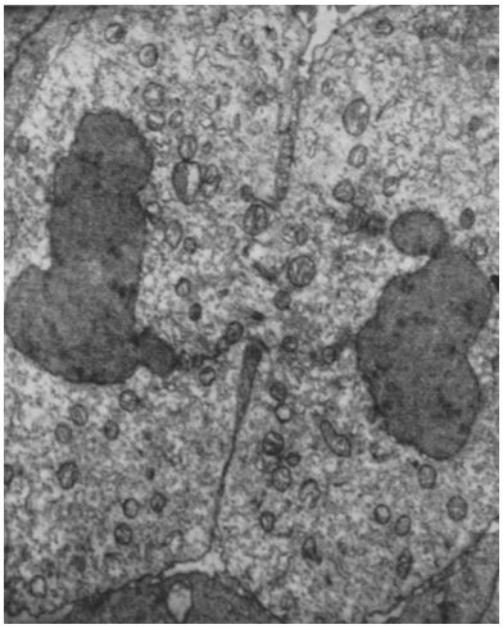

FIGURE 8

Cell of Walker 256 carcinoma. A long cleavage furrow extends from the telophase bridge to the margin of the cell. × 7000.

arranged and have developed to about the same degree in each cell. Each cisterna consists of a double membrane reaching almost from the equatorial surface of the nucleus to the cleavage plane. It then passes axially almost to the mid-body, where it curves again toward the nucleus, and this part of it encircles the central spindle. Numerous perforations exist in the cisternae, especially on the surface facing the spindle. We are indebted to Dr. Keith Porter for the suggestion that these cisternae are a part of the ER. In the erythroblasts the membranes do not have RNP particles on their outer surfaces, but they do in the cells of Walker tumor.

Although fewer tumor cells than erythroblasts have been observed in the critical stages of cleavage, the images indicate that the process is essentially similar. In Fig. 7 a telophase cell of mouse leukemia displays a short row of vesicles on the cleavage plane. Unfortunately, this cell has not been sectioned through the mid-body. Because of their large size, the cells of Walker 256 carcinoma are rarely found to be sectioned parallel to the long axis and also through the mid-body, but one such cell is shown in Figs. 8 and 9. In the short intercellular telophase bridge are rather widely separated strands of the dense material of the mid-body. A long cleft extends from the telophase bridge to the margins of the cell. The interesting feature of this cleft is that the lining of the new plasma membrane is thrown into a great number of microvillous projections. Other parts of the plasma membrane of this cell show few microvilli. The appearance suggests to us the redundant plasma membrane which would be expected to result from the fusion of a series of vesicles on the equatorial plane. As vesicles grow and then fuse together to form a continuous membrane, the surface area of the latter will necessarily be greater than that of a smooth membrane extending between the same points (telophase bridge to cell margin). It is also possible that an unknown factor promoting the growth of the

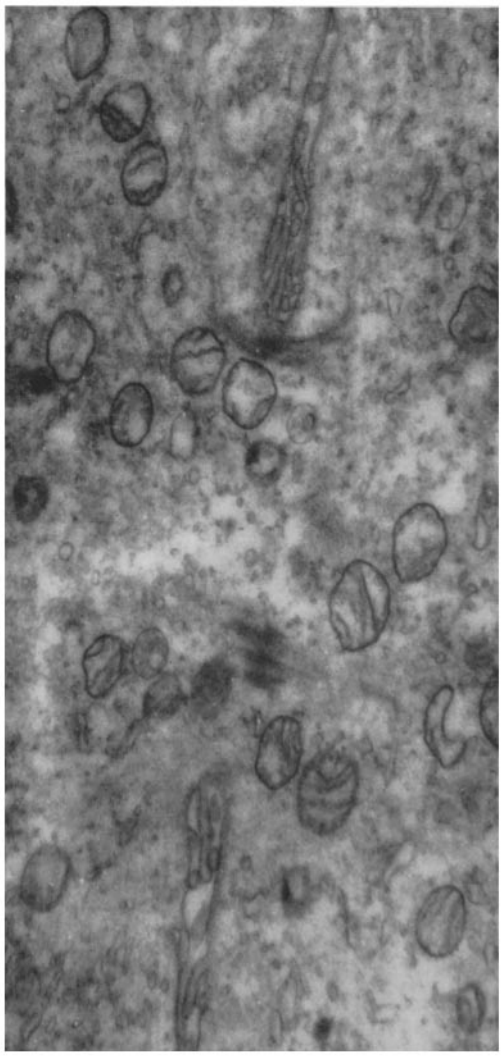

FIGURE 9

Telophase bridge and deep margin of the cleavage furrow in another section of the cell shown in Fig. 8. The section passes through two pieces of dense, fibrillary material, which are parts of the mid-body. Numerous microvilli project into the furrow. × 22,000.

vesicles might likewise promote the growth of microvilli after fusion of the vesicles.

DISCUSSION

The formation of vesicles on the equatorial plane has been observed in telophase of *Allium* root tip (2, 11), and their development proceeds in a centrifugal direction (11). The suggestion was made by David (3) that the intracellular synthesis of new plasma membrane of the cleavage furrow may occur in mitotic cells of rat embryonic liver. The formation of the new membrane was said to begin at an indentation in the old one and to involve the participation of granules extending to a vacuole deeper in the cell. We are grateful to Dr. Donald P. Costello for bringing to our notice a description by Wilson (14) of cleavage in fish eggs and eggs of *Thalassema*, in which the formation of vacuoles on the equatorial plane was seen. More recently, the development of new cleavage membrane from vacuoles has been suggested as the mechanism of cleavage in sea urchin eggs (9). In dividing newt cells localized cortical growth, in the form of a double layer of cytoplasm, was observed to be the basis of a preformed cleavage plane (12).

The mechanisms of cleavage suggested for plant cells (2, 11), rat embryonic cells (3), fish and *Thalassema* eggs (14), sea urchin eggs (9), newt eggs (12), and the cells of our study, although different, have in common the idea that structures or materials with a transverse orientation exist on the equatorial plane, and that before the time of the appearance of the cleavage furrow a structural basis for the new membrane already exists. This is quite different from the concept, which seems to be widely accepted, that the cleavage of mammalian cells involves the contraction of a band of cytoplasm, constricting the cell at the equator. If published photographs, especially phase contrast photographs, of mammalian cells in early telophase are examined with the idea in mind of an equatorial plane of vesicles, at least some suggestion may be obtained of transversely oriented material. Thus, Hughes and Swann (5), studying chick fibroblasts in tissue culture with polarized light, noted a birefringent line running perpendicular to the long axis of the cell during cleavage. They did not commit themselves in regard to its significance, but, in our opinion, it may possibly represent the cleavage vesicles. Similarly, in cine-films of dividing cells in tissue culture, one can interpret cleavage as the opening up of a preformed membrane, rather than the constriction of the old one.

An interesting observation, which might be consistent with our finding of a vesicle surrounding the mid-body, was made by Mota (8), studying grasshopper neuroblasts. For some reason, not known to him, certain cells underwent karyo-

kinesis without cytokinesis, and in these he observed that the middle of the spindle was encircled by a ring of material. We wonder if this material might have been a vesicle of the type we have described.

That the position of the cleavage furrow is determined by the mid-point of the spindle is well established for certain types of cells. Kawamura (6) found in grasshopper neuroblasts that, regardless of how the spindle was displaced by microdissection, the cleavage furrow always formed around its middle part. He postulated that the cell surface responds, by undergoing cleavage, to some substance formed by the spindle. On the other hand, the cleavage of sea urchin eggs proceeds normally when the spindle is removed during anaphase, and the position of the cleavage furrow is determined by the position of the spindle before anaphase, but not later (4).

According to our observations, the process of cleavage involves three steps, which may not be directly related. These are: (1) moderate narrowing of the middle of the cell during late anaphase and early telophase, (2) development of a cleavage furrow by the fusion of vesicles on the equatorial plane, and (3) parting of the intercellular telophase bridge.

The first stage represents a progression from the cylindrical shape of the cell in anaphase. It corresponds to the period of the laying down of the major part of the nuclear membrane; when it terminates, the chromosome masses are in the shape of thick discs positioned transversely in each daughter cell. The narrowing of the middle part of the cell is considered to reflect the natural conformity of the plasma membrane to the size, and perhaps rigidity, of the cell contents. If we assume that the telophase nuclei, which are very dense in electron micrographs, have greater rigidity than the cytoplasm, we would expect the plasma membrane to be somewhat wide at the level of the nuclei and narrower between them.

At this stage the mid-body is already present. The development of the cleavage furrow then proceeds centrifugally, beginning with a vesicle (perhaps several vesicles which fuse together) close to the mid-body. Whether the mid-body produces some substance which influences the adjacent cytoplasm to become converted into a membranous vesicle is, of course, unknown, but the spatial and temporal relationship of the first vesicle to the mid-body seems clear.

The first vesicle seems to be fundamentally different from the other vesicles which lie in the equatorial plane. In many images it is the only vesicle present, and this suggests that it forms first. It is considerably larger than the other vesicles when they do appear, and its profile has a different shape. On the available evidence we cannot deduce the role of the first vesicle in giving rise to the other vesicles of the equatorial plane. The fact that its membrane may be continuous with the membrane of the other vesicles does not, of course, establish the origin of the one from the other.

A significant point, it seems to us, is that, at the time of the appearance of the first vesicle, there is no extensive development of the membranes of the ER near the cleavage plane. In images which show either a row of discrete vesicles or the continuous membrane of the early cleavage furrow, cisternae of the ER are found in relation to the central spindle and the cleavage plane. These cisternae would appear, from their distribution, to have a role in forming the vesicles. Perhaps vesicles arise by a process of pinching off, similar to that observed in the development of pinocytotic vesicles from plasma membrane (10). It seems likely that these cisternae are comparable to those which were seen by Porter and Caulfield (11) extending toward the cleavage plane and which, they believed, gave rise to vesicles there.

The final stage of cleavage, that of separation in the telophase bridge, remains to be studied from the standpoint of the participation of the mid-body and the ER or other membrane-forming structure. It is possible that, here also, the separation is effected by the fusion of tiny vesicles to one side of the mid-body (1). In the living cell the bridge is observed to snap and the mid-body goes to one of the daughter cells (5). The fate of the mid-body in the cell receiving it is unknown.

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