THE FINE STRUCTURE OF MITOSIS IN RAT THYMIC LYMPHOCYTES

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ABSTRACT

The fine structure of rat thymic lymphocytes from early prophase to late telophase of mitosis is described, using material fixed at pH 7.3 either in 1 per cent OsO_4 or in glutaraldehyde followed by 2 per cent OsO_4. The structure of the centriolar complex of interphase thymocytes is analyzed and compared with that of centrioles during division. The appearance of daughter centrioles is the earliest clearly recognizable sign of prophase. Daughter centrioles probably retain a secondary relation to the primary centriole, while the latter appears to be related, both genetically and spatially, to the spindle apparatus. The nuclear envelope persists in recognizable form to help reconstitute the envelopes of the daughter nuclei. Ribosome bodies (dense aggregates of ribosomes) accumulate, beginning at late prophase, and are retained by the daughter cells. Cytokinesis proceeds by formation of a ribosome-free plate at the equator with a central plate of vesicles which may coalesce to form the new plasma membrane of the daughter cells. Stages in the formation of the midbody are illustrated.

The fine structure of the mitotic process has been extensively studied in plant cells (30, 38) and in a variety of non-mammalian forms (1, 13, 17, 36, 48), but detailed descriptions of mitosis in mammalian cells are not numerous (7, 8, 11, 19, 33, 40). Since the proliferative activity of the thymus has been extensively analyzed (21), and because of the current widespread interest in the thymus as it relates to immune mechanisms (25), a detailed study of mitosis in the thymus should be of value. We have begun such a study in connection with our investigation of the fine structure of thymocytes (27). Unfortunately, *in vivo* studies of this material do not permit preselection of a given stage of mitosis for serial sectioning, and chance plays a large part in the selection of cells for study. Designation of particular stages in the mitotic cycle must be considered tentative, for each cell is represented by one or, at best, a few adjacent sections, which greatly handicaps the study of three-dimensional features and of dynamic events. Over 200 mitotic cells have been photographed, however, which permits a preliminary description of the basic features of mitosis in this situation.

MATERIALS AND METHODS

The thymuses of 8 rats, ranging in age from 1 to 3 weeks, were sampled. Tissues were fixed in 1 per cent OsO_4 in balanced salt solution ($CaCl_2$, 0.008 M) buffered between pH 7.2 and 7.4 with Veronal (28) or in 3 per cent glutaraldehyde at pH 7.3 in Sörensen's phosphate buffer and subsequently treated with 2 per cent OsO_4 (37). Embedding was done in Epon 812 essentially according to Luft (23), and staining in uranyl acetate (47) or a combination of uranyl acetate and lead citrate (31). Sections were examined on 300-mesh copper grids without other support, in a Hitachi HU-11 electron microscope.

RESULTS AND INTERPRETATION

We have described the fine structure of the inter-

phase thymocytes in a previous paper (27). Although there is considerable variation in the nuclear pattern and the extent of cytoplasm, Fig. 1 illustrates the general features of interphase thymocytes. The absence of a distinct nucleolus is the rule, although some of the larger cells show a region of finer granules, more compactly arranged. In all cases the nucleus contains masses of dark granular material which is extensively associated with the nuclear envelope. A moderate number of mitochondria, very rare elements of the endoplasmic reticulum, numerous ribosomes, a compact Golgi apparatus, and a pair of centrioles are found in all the thymocytes regardless of size, although they are not, of course, seen in each cell profile. The centrioles are distinctly separate, in an angular but not necessarily perpendicular relation to each other, and consist of nine groups of tubules arranged to form a cylinder (Figs. 2 to 7). The number of tubules in each group is at least two and sometimes three, as reported by Bernhard and de Harven (3). The tubules are embedded in a matrix, which gives the whole cylinder a diameter slightly larger than that of the ring of tubules. In a longitudinal profile, as in Figs. 4 and 5, it can be seen that similar material is present within the cylinder also, but only for about half its length. One end is empty for approximately ⅓ the length (Fig. 1), and usually a short segment at the opposite end is also empty. A channel with dense walls (Fig. 7) pierces the substance longitudinally in the central axis.

The dimensions of the centrioles vary considerably among cells, but not between the centrioles of the same cell. Based on the measurements of 60 centrioles, the diameter of the ring of tubules in cross-sections ranged from 165 to 210 mμ and the lengths from 300 to 450 mμ. These values agree quite well with those reported for the rat thymus by de Harven and Bernhard (9).

Surrounding many of the profiles of centrioles structures are observed which were first described in human leukocytes by Bessis and Breton-Gorius (4), and later in spleen of young chickens by Bernhard and de Harven (3), who termed them pericentriolar bodies or satellites. Similar structures surrounding the centrioles of jellyfish have been reported by Szollosi (43). They are dense bodies without sharp boundaries (Figs. 3, 7), about half the diameter of the centriole, and connected to it by a bridge of less dense material. The bridges lie in a plane perpendicular to that of the axis of the cylinder (Fig. 4) and slightly oblique to a radial direction (Fig. 3). They relate to the interspaces between the tubules (Fig. 3), lie opposite the region of the cylinder occupied by the central dense material (Fig. 4), and are composed of similarly appearing substance. Although there appear to be one body and one bridge for each group of tubules, we have not seen nine complete satellites in a single profile. We have seen as many as four (Fig. 3) in a profile which was nearly at right angles to the centriole axis. Since there was a slight tilt in one direction, the other five could lie slightly above or below the plane of section. In another instance (Fig. 6), nine small projections can be counted but the pericentriolar bodies are not so complete as in many other cases.

It is perhaps significant that in no case did we see well formed pericentriolar bodies on both centrioles of a pair. We have often failed to see any part of the pericentriolar complex in profiles

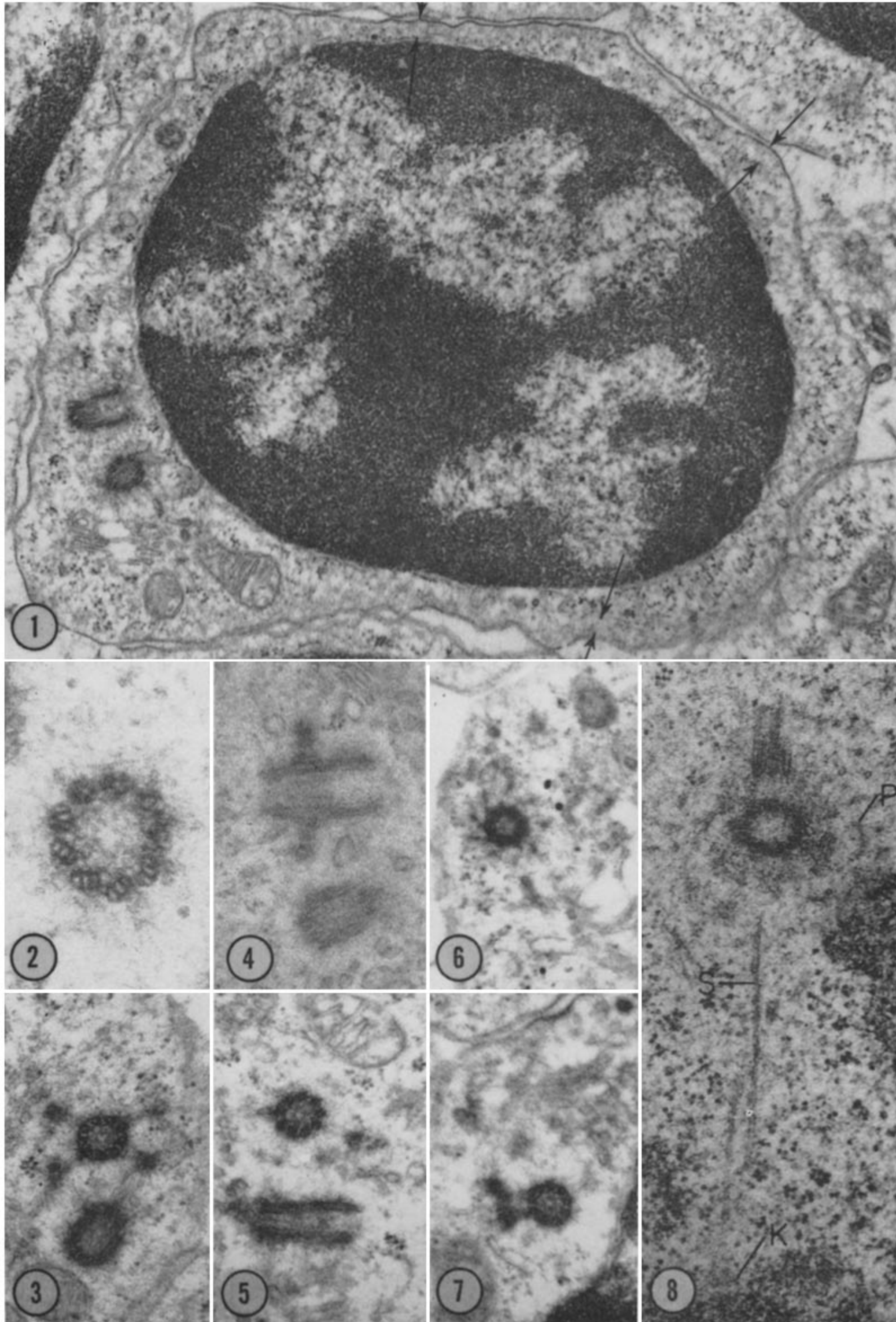
All the illustrations represent rat thymocytes, or portions thereof, prepared as indicated under Materials and Methods. All cells were fixed in 1 per cent OsO_4 except that shown in Figs. 11 and 12. Only certain structures of especial interest are indicated. Minimal essential data are included. For detailed descriptions, see text.

FIGURE 1 Small thymocyte in interphase. Note ribosome-free zone between arrows. × 26,500.

FIGURE 2 Centriole of another interphase thymocyte. × 96,000.

FIGURES 3 to 7 Several centrioles of interphase thymocytes. Compare with Figs. 8 and 13 showing centrioles of mitotic cells. × 36,000.

FIGURE 8 Pair of centrioles of a thymocyte in prophase. Spindle tubule (*S*), a kinetochore (*K*), and suggestions of a pericentriolar body (*P*) are indicated. × 37,000.


1
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that would appear well suited to its demonstration. The frequency of this occurrence suggests that not all interphase centrioles possess these bodies, but the frequency of their presence on one of the pair suggests that they are not of a brief appearance at the end of interphase. The most likely interpretation is that the bodies develop during interphase but are fully formed on at least one of the pair of centrioles before prophase is initiated. The possibility that the two centrioles are not entirely alike is further suggested by the frequent occurrence of a more pronounced, relatively empty zone around one centriole of the pair than around the other. It should also be emphasized that in no case, in a clearly interphase cell, were more than two centrioles seen.

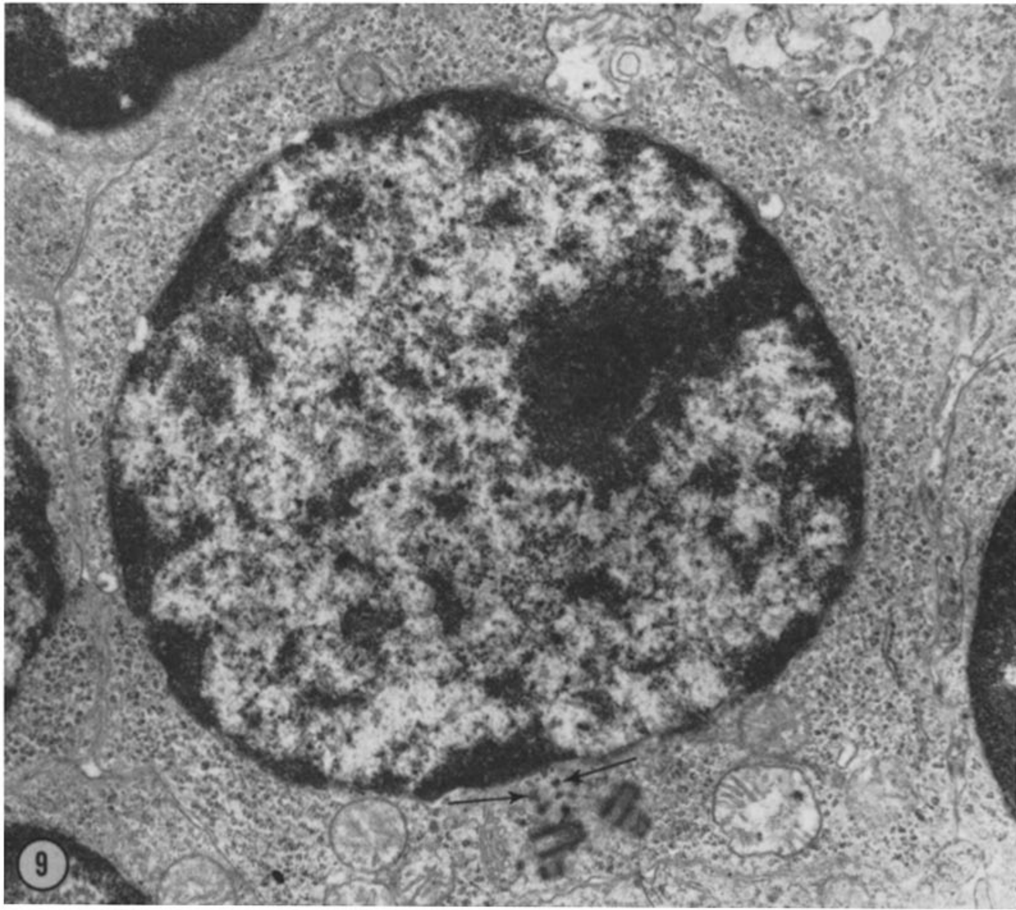
Prophase

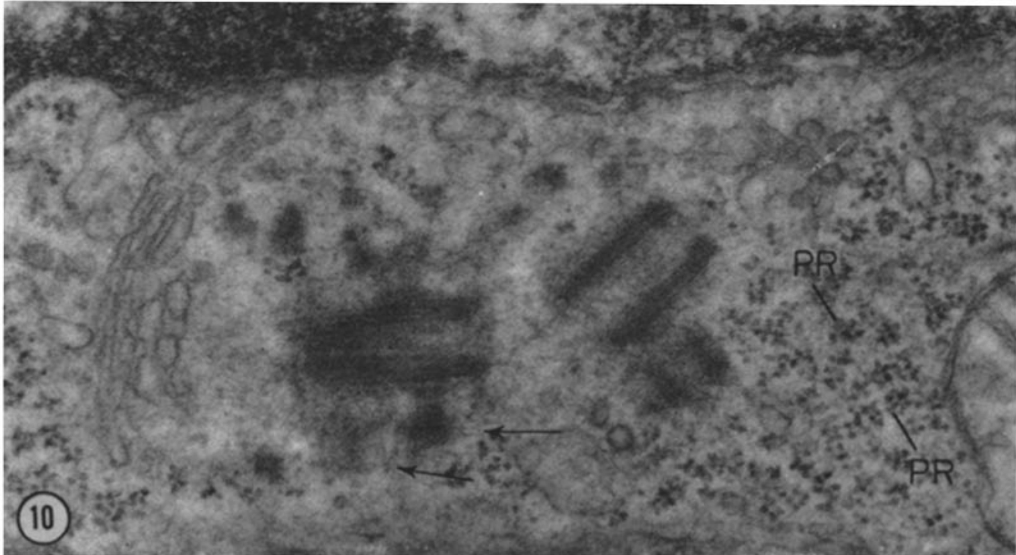
The earliest changes in nuclear pattern which indicate the beginning of prophase are difficult to characterize, especially against the background of variation in the resting cells. The nucleus of Fig. 9, however, appears to have less granular material massed at the nuclear membrane and a number of aggregates of this material throughout the nucleus. The most striking feature of this cell is the pair of centrioles, each member of which has a short structure related to one end, at right angles to it, and consisting of parallel tubular elements. We have interpreted these as budding centrioles, as described by others (3, 16). That they are not pericentriolar or satellite bodies is shown by the tubular elements in the buds and by the presence of typical pericentriolar bodies at the other end of one of the centrioles (Figs. 9, 10). These two figures represent sections through the same pair of centrioles at sufficiently different levels that a bud, present in one (Fig. 9), is nearly missed in the other (Fig. 10), but still no pericentriolar bodies or connecting bridges can be seen on this second centriole in either figure. This supports the possibility that only one of the centrioles of the resting cell has a completely developed pericentriolar organization. Another feature of this cell which seems to be characteristic of early prophase is the unusually heavy concentration of ribosomes in the cytoplasm, largely in the form of small clusters (Fig. 10). These clusters may correspond to the polyribosomes or polysomes found in reticulocytes (45) and other cells. In addition, the earliest evidence of tubules of the dimensions of spindle fibers can be seen in relation to one of the pericentriolar bodies in Fig. 10. In only one other instance did we find centrioles with buds. The nucleus of this cell also was suggestive of very early prophase.

At a slightly later stage of prophase (not illustrated) the aggregation of nuclear densities was more distinct and the nuclear envelope, which was still intact in Fig. 9, was seen to have major gaps. The region near the Golgi membranes, hence presumably near the position of the centrioles, was especially deficient. At a still later stage of prophase, as represented by Fig. 11, the gaps are wider, but a large part of the nuclear envelope remains. A feature of this stage is the formation of duplications of this envelope, possibly the same phenomenon referred to as "stacking" by other authors (2). One centriole is present, with its axis at approximately right angles to the apparent axis of the developing spindle. The pericentriolar material in prophase cells (Figs. 8, 12) has a different character than in the resting cell. In Fig. 8, there is a broad zone of amorphous substance immediately outside the centriolar tubules beyond which spindle fiber tubules can be seen projecting a short distance. Fig. 12 shows the pericentriolar material arranged in part as flame-shaped projections, with broad bases and slender tips. Spindle fibers, in the form of tubules about 20 to 25 mμ in diameter, are seen in relation to at least one slender tip. Kinetochores are also seen where these tubules attach to the chromosomes, and resemble, in general, those demonstrated by

FIGURE 9 Thymocyte in very early prophase. Note centriolar buds on each one of the pair of centrioles, and a pericentriolar body attached to the left centriole. Several other similar bodies (arrows) lie in the vicinity of this centriole. $\times$ 17,000.

FIGURE 10 Enlarged view of the centrioles of the same cell as that in Fig. 9, but in an adjacent section. Polyribosomes (*PR*) and early microtubule formation at arrows. $\times$ 54,000.




RR
PR

Bernhard and de Harven (3) and Robbins and Gonatas (33). The ribosomes at this stage, rather than forming many small, regular clumps, show a tendency to gather in larger, more irregular aggregates.

Metaphase

The exact separation of prophase and metaphase is not possible in our material because of the random nature of selection. Figs. 13 and 14 have been chosen to represent the metaphase stage since there seem in each case to be an equatorial concentration of the chromosomal material and a fairly distinct indication of a spindle at right angles to this plane. Only one pair of centrioles is apparent in each case, but the approximate location of the other pair can be surmised from the spindle fiber concentration. One of the pair of centrioles, hereafter called the primary centriole, is at right angles to the axis of the spindle, but the secondary centriole is not in any consistent relationship to this axis. It is, however, at right angles to the primary centriole, but apparently can vary from being pointed toward the pole, as in Fig. 14, to a direction inside the spindle cone, as in Fig. 13. Both centrioles have densities surrounding them, but the density around the secondary centriole is confined to one end where it may well be considered part of the primary complex, while the inside of the cylinder of the secondary is relatively empty (Fig. 13). The spindle fibers relate more specifically to the primary centriole. The two centrioles are not so widely separated as in the resting cell, the interval between them being fairly constant and equal to approximately half the diameter of the centriole (Figs. 8, 13, and 14). This distance is only slightly greater than that which separates the original centriole bud from its parent at early prophase (Figs. 9, 10).

The tendency toward aggregation of ribosomes, noted earlier, appears somewhat more marked in these cells. They both show extensive remains of the nuclear envelope, and stacking of these membranes is clearly evident in Fig. 14. The sudden appearance of these duplicated membranes suggests that they may arise at the time of the opening up of the nuclear membrane by a folding over of portions of the envelope. In the cell of Fig. 13, however, there is an almost complete investment with a nuclear envelope remnant but no evidence of stacking. It is possible that the stacking phenomenon is not characteristic of all cells. The cell of Fig. 13, although apparently sectioned through the center, measures only 6 μ in diameter, which is about the lower limit of size observed among mitotic cells in our material. The relative scarcity of cytoplasm in these small thymocytes may account for the absence of stacking, in that insufficient space is allowed for folding over to occur.

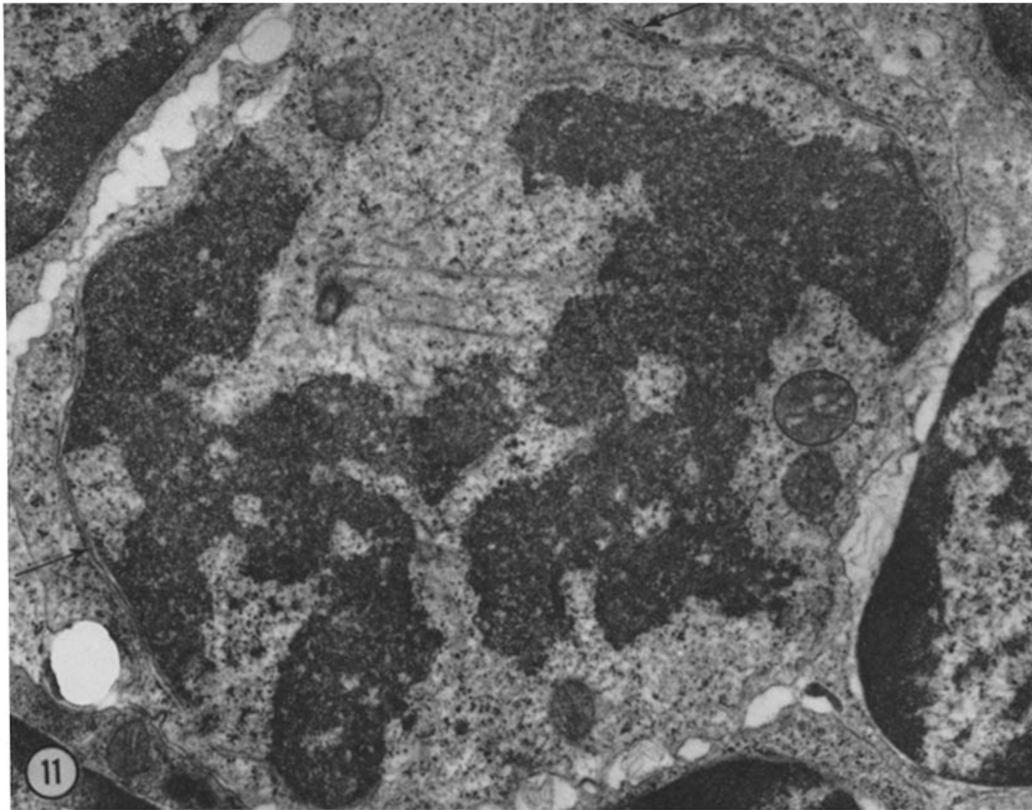
The kinetochore, demonstrated more clearly than previously, consists of a density without internal structure, separated from and paralleling the surface of the chromosome. Spindle fibers are seen to embed in this density (Fig. 14). At one point the kinetochore appears in the shape of a ring. Another significant feature of this stage is the frequent occurrence of swollen crista membrane spaces, as seen in several of the mitochondria in Figs. 13 and 14. This effect may be an artifact of preparation, for similar appearances are noted in interphase cells, but a count of 100 cells of each type revealed that only 20 per cent of interphase cells show it, while nearly 80 per cent of mitotic cells, and virtually all cells in late stages of mitosis, show some degree of swelling of these spaces.

Anaphase

In anaphase, as illustrated in Fig. 15, the aggregation of ribosomes is more marked than during metaphase. The mitochondria and the ribosome masses apparently may occupy positions in the center of the cell, presumably within the mitotic apparatus, although spindle fibers were not demonstrated in this preparation. We have seen mitochondria within the mitotic apparatus in many other cells. Investment of the daughter

FIGURE 11 Thymocyte in prophase. Note duplication of nuclear membrane at arrows. Fixed in glutaraldehyde followed by OsO_4. $\times$ 13,000.

FIGURE 12 Enlargement of the region of the centriole of Fig. 11. Note small ribosome aggregates (*R*), flame-shaped projections of pericentriolar material (*F*), and kinetochores (*K*). $\times$ 39,000.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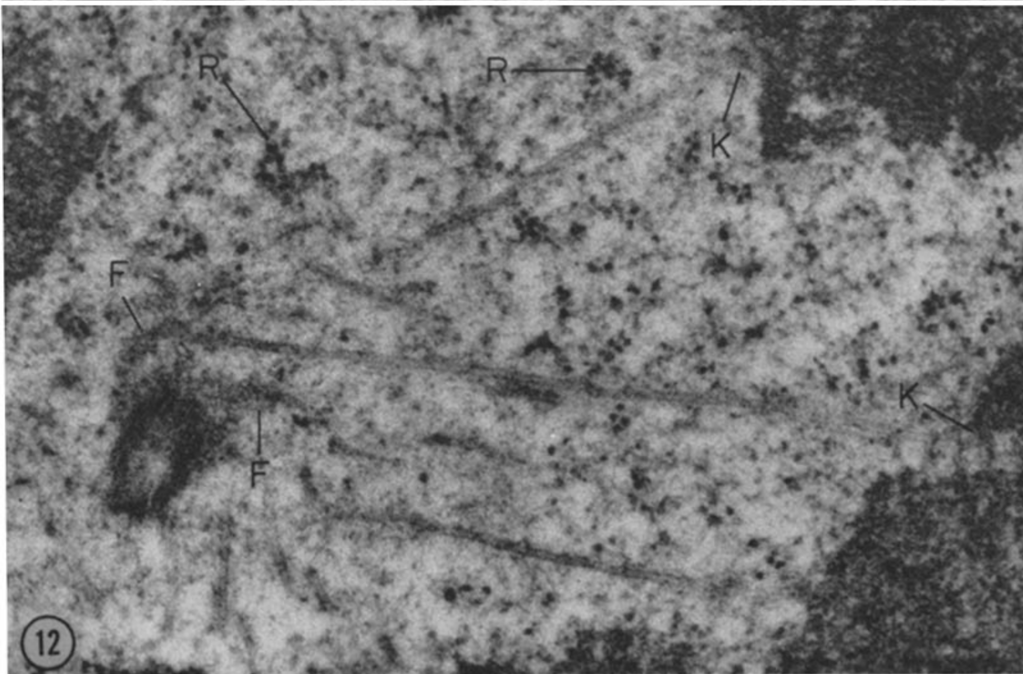
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12

nuclei has begun, and, as other workers have reported (33), it is first noticeable on the polar aspects of the chromosomes.

Typical nuclear pores are evident in the oblique section of the nuclear envelope. Along one side of the cell a line of vesicles and elongated cisternae indicates the remnants of the nuclear envelope, and in several places it appears that these membranes are providing the material for the new nuclear envelope. Portions of nuclear envelope caught between the condensing chromosomes indicate that the chromosomes are at least partially invested with membrane before they condense together, as reported by others (2, 7). At one point the new nuclear investment appears in part to be four-layered, like the limited stacks of prophase, but at one end the stack seems to be unfolding, and is coated with ribosomes. At a slightly later stage of anaphase (Fig. 16), the equatorial region has narrowed and shows in its center a few spindle fibers with dense areas, the first evidence of the formation of the midbody. There is no other specialization of the equatorial region. Neither thickening of the ectoplasm at the neck region nor "bleb" formation, as reported for the HeLa cells at this stage (33), are evident in this cell, or in any other we have examined. An adjacent interphase cell does present a bleb, however, which is typical of many such cells throughout the thymus, as previously reported (27).

Telophase

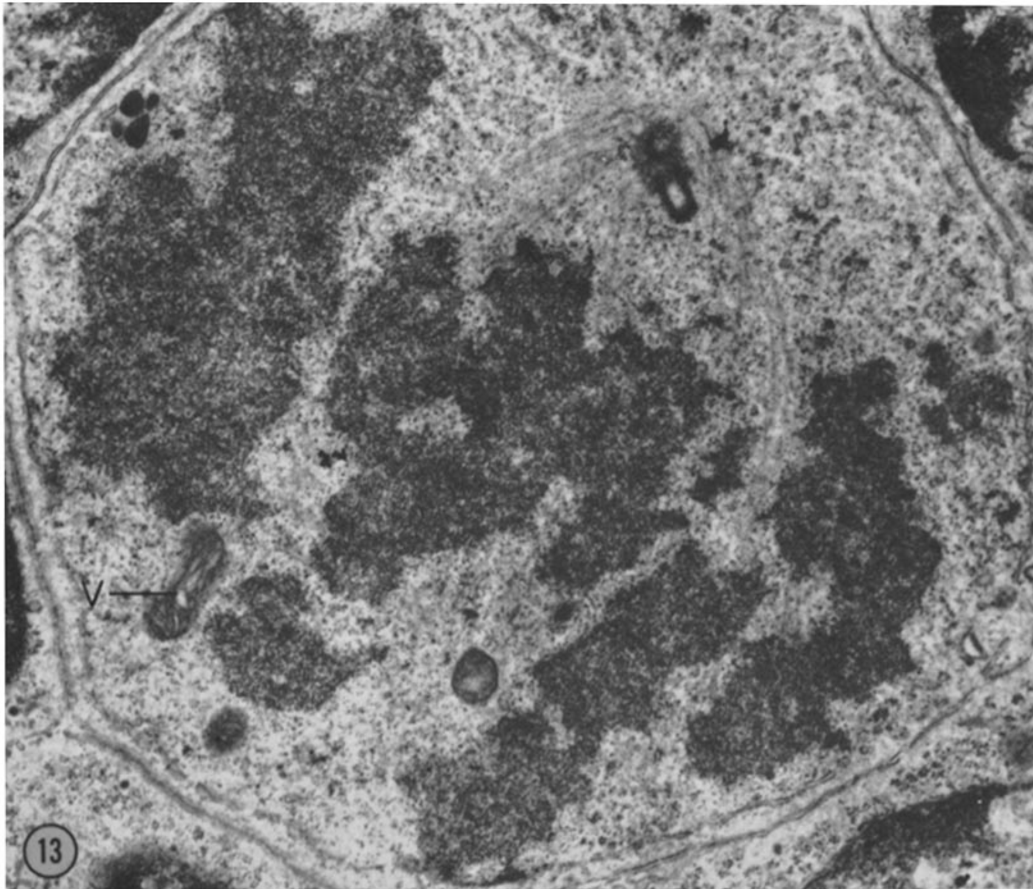
Figs. 17 to 21 illustrate the mode of separation of the daughter cells. Fig. 17 shows the equatorial region of a cell in telophase, sectioned in a plane lateral to the central axis. Along the line of separation between the two daughter cells is a band of ribosome-free cytoplasm, and in this band is a row of vesicles. This band is identical in appearance and continuous with a differentiated band of cytoplasm beneath the plasma membrane. We have commented previously (27) on the existence of this band in interphase thymocytes. In this cell also, regions on a daughter nucleus are covered by stacked membranes of the type which form during prophase at the time of breakdown of the nuclear envelope. A vesiculated equatorial plate is also seen in Fig. 18, which shows in addition an advanced stage of midbody formation at the line of separation, associated with a substantial bundle of spindle fibers crossing between the two cells. The angular relation of this bundle to the axis joining the two cells is not unique to this pair, but is the rule in those cells we have studied, and the location of the midbody to one side is similar to the situation illustrated, although not emphasized, by Buck (7). Apparently the separation of the daughter cells is complete on one side, and on the other the equatorial plate of ribosome-free material is separated down the middle by a row of vesicles which have not yet fused to form the separating furrow. Ribosome aggregates and swollen crista membrane spaces in mitochondria are also noted in this cell. Mingling with the spindle fibers at one point is a group of membranous structures resembling the smooth-surfaced endoplasmic reticulum. Several authors have described membrane elements lying between the spindle fibers (6, 30), but this is the only indication in our material of such an occurrence. Fusion of the equatorial vesicles to form the plasma membranes of the daughter cells is indicated in Fig. 19 which shows, in addition, especially clear instances of nuclear pores. A later stage of midbody formation is illustrated in Figs. 20 and 21. The fibers are seen only in the central density and extending a short distance into one of the daughter cells. There is no indication of a movement of the dense material to the surface of the narrow neck of cytoplasm, as happens in the erythroblast at this stage (7).

DISCUSSION

In selecting cells to represent the various stages of mitosis, we have depended primarily on the pattern of distribution of the densely osmiophilic material of the nucleus. The term chromatin is

FIGURE 13 Thymocyte in prophase or early metaphase. Note vacuolated crista membrane spaces (V) in mitochondria $\times$ 18,000.

FIGURE 14 Part of a thymocyte in metaphase. Note the vacuolated crista membrane spaces (V) and the kinetochores (K), the lower one with a circular profile. $\times$ 24,500.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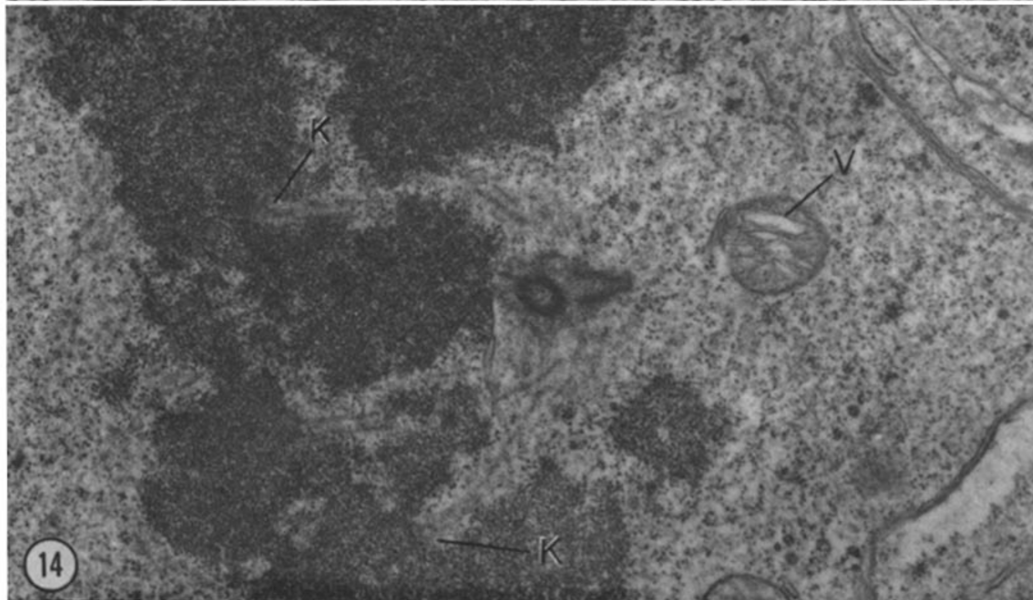
K
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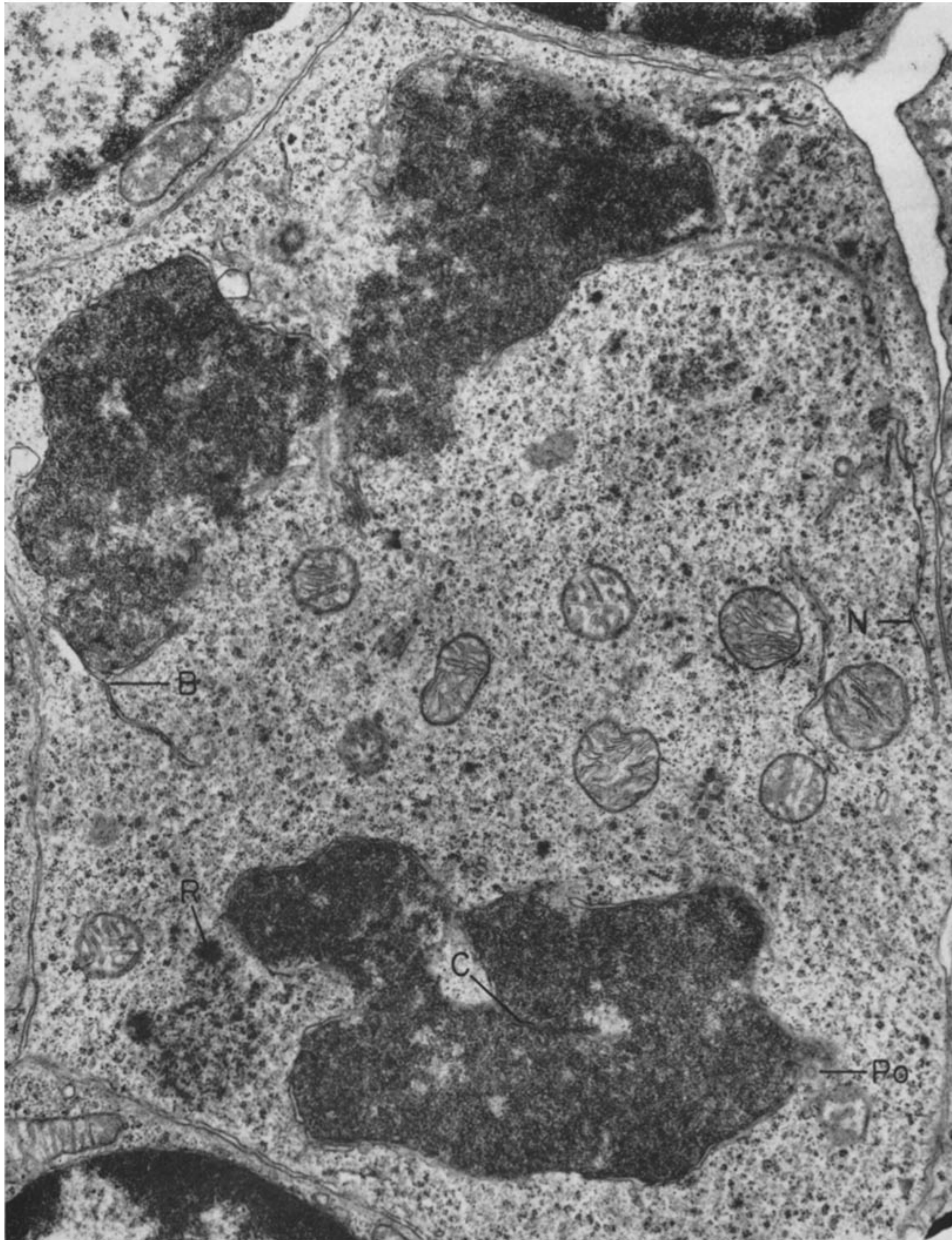


FIGURE 15 Thymocyte in late anaphase. Note ribosome body (*R*), nuclear pore (*Po*), and nuclear membrane remnants (*N*). At one point (*B*) the outer pair of membranes of a doubled nuclear envelope may be separating from the inner pair of membranes, and at (*C*) a portion of the nuclear membrane is caught among the condensing chromosomes. × 14,000.

widely used by electron microscopists in referring to this material, but it has never been properly demonstrated that the osmiophilic granules are coextensive with the substance which stains heavily with basic dyes in preparations for light microscopy. Bloom and Leider (5) have shown that the element in the chromosomes of newt fibroblasts, which is so dense in OsO_4-fixed material, is not affected by an ultraviolet beam which destroys the DNA in the irradiated portion of the chromosome. We feel, therefore, that caution should be exercised in the use of the term chromatin. However, in the absence of other criteria, we have judged that when this osmiophilic material shows a pattern similar to that of the chromatin in early prophase as seen with the light microscope, we are dealing with an early prophase nucleus. Our analysis of the chronology in our material has been greatly aided by the work of Robbins and Gonatas (33), where the various stages were selected *in vivo* by phase-contrast microscopy prior to preparation for electron microscopy. In general we have found the sequence of events quite similar to those they report, as well as to the process as seen in erythroblasts by Buck (7), and in lymphocytes stimulated by phytohemagglutinin (19). Since certain details of the participation of various cell organelles in mitotic process may perhaps be illuminated by events in our material, we shall comment briefly on these.

Centrioles

The frequency of finding two, and only two, centrioles in resting cells makes it quite likely that this is the usual condition in thymocytes, as it appears to be in other cells (3). If, as Mazia (24) has postulated, there are potentially four centrioles after late telophase, we presume that the replicated daughters are not yet large enough to be visualized. On the other hand, mitosis in thymocytes may differ from that in sea urchin eggs, and replication may not occur until the time of budding. Bernhard and de Harven (3) and Gall (16) have suggested that the pericentriolar bodies might have some role in the budding of new centrioles, but this does not seem likely. At the stage of budding, evidence of the satellites can be seen on the same centrioles from which the bud is developing, in both our material and that illustrated by these authors.

We conclude that budding takes place as the earliest recognizable event of the mitotic process, since we have found it only twice in over 150 profiles containing one or more centrioles, and in these two cells there were suggestions, but no unequivocal signs, of early prophase. This is, of course, not a surprising conclusion, as an exactly similar finding was reported for the spermatocytes of the hagfish by Schreiner and Schreiner (39) 60 years ago. We also find, as they did, that the daughter centriole continues its development during mitosis, and probably is not fully mature until anaphase or later. The older centriole, which we have termed the primary one of the pair, apparently acts as the focus of the spindle and maintains a relatively constant orientation perpendicular to the spindle axis, while the daughter, or secondary, centriole is still in the process of maturation. Serial sections are needed to establish this relation with certainty.

How long do the members of the new pair remain different in structure? We have seen an apparent primary-secondary relationship as late as anaphase, but our material at later stages is largely lacking in centrioles. It is possible that even during interphase the two centrioles are not entirely similar, since we have never demonstrated "typical" pericentriolar bodies on both centrioles of a pair. Bernhard and de Harven (3) also have commented on the inconsistency of their finding of pericentriolar bodies. It is not clear whether the small projections resembling a pin wheel in Fig. 6 represent a different structure, or an earlier stage of the same structure as do the more massive appendages seen in Figs. 3, 4, and 7. Figs. 9 and 10 suggest that, as late as the stage of budding, one of the centrioles does not possess a well developed pericentriolar arrangement. Insofar as these satellites are essential to spindle formation, it would appear that the two pairs of centrioles as they enter prophase are unequally equipped. If this is so, and if differentiation were to occur during mitosis, this lack of identity of the centriole pairs might be a factor in the process.

Nuclear Envelope

In living mammalian cells under phase-contrast microscopy, disappearance of the nuclear membrane is an early and dramatic event of prophase (15). In thymocytes, however, it would not be accurate to say that the nuclear envelope disappears completely at any stage, as seen with the electron microscope. In late prophase or early metaphase, a complete ring, broken only by what

might be especially large pores, is still seen in many cases, and even at anaphase large segments can be recognized. It seems very likely that substantial portions of the envelopes of the daughter nuclei are supplied by these remains of the old nuclear membrane. This hypothesis is supported by the appearance of patches of four-layered membrane on the daughter nuclei, which are similar to the duplications that appear at prophase during the partial disruption of the envelope. Our material does not establish this with certainty, for the extent to which the endoplasmic reticulum may be involved cannot be determined. There is very little of this latter element in thymocytes. Since it has been proposed that the endoplasmic reticulum may originate from the nuclear envelope (46), the events at mitosis can be interpreted as illustrative of a fundamental similarity in these two structures, and the extent to which original nuclear envelope is involved in daughter nuclear membrane becomes less significant. Perhaps the thymocyte is more like the ameba (36) which has only a brief and very limited disruption of the nuclear membrane with obvious reutilization in reconstituting the daughter nuclei.

Spindle Fibers

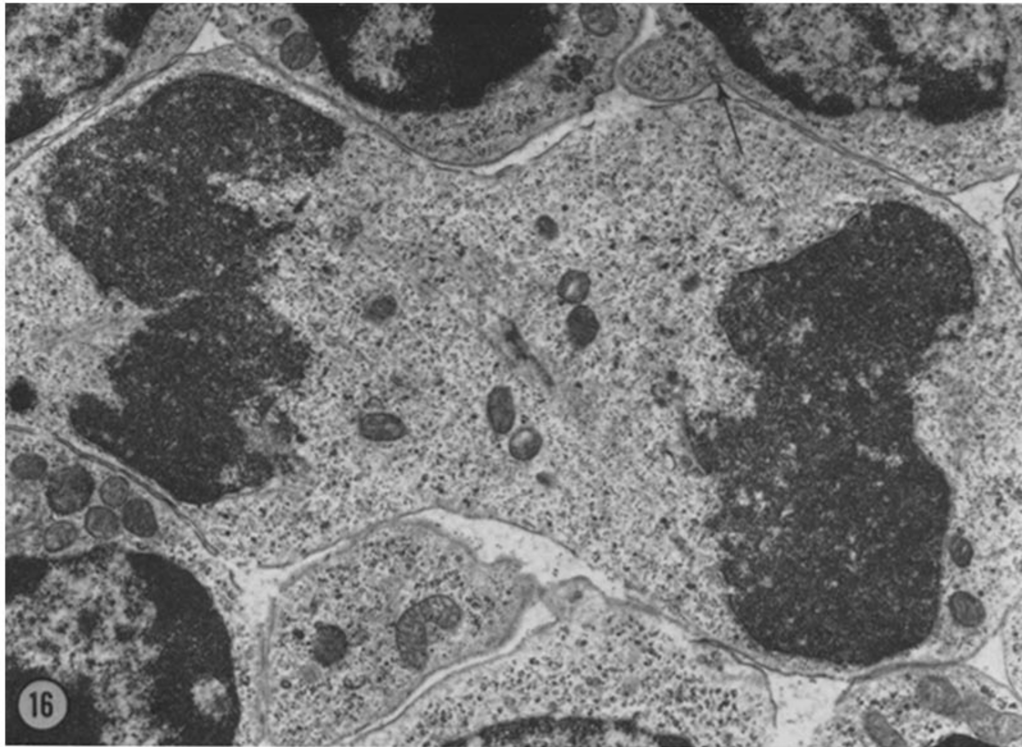
Contrary to the findings of others (10, 22, 33, 41), we find little evidence of tubular structure in the cytoplasm prior to very early prophase, in material fixed in either buffered OsO_4 or glutaraldehyde. Much of the material examined was fixed in OsO_4 at pH 7.3, and although there was a content of bivalent ions (0.008 M, $CaCl_2$) the conditions may not have been optimum for preservation of spindle tubules (18, 35). They were, however, visualized sufficiently to suggest certain interpretations. The special relation of the spindle fibers to the centrioles and to the satellite bodies suggests that the centrioles may have a role in their formation as others have suggested (9, 10). In support of this are the following considerations:

1. The tubule groups in the centrioles of interphase cells are embedded in an amorphous substance, and similar material is seen within the cylinder, concentrated in the region of attachment of the satellites.
2. The primary centriole of mitosis is surrounded by a broad zone of similar-appearing amorphous substance which is continuous with the substance within the cylinder.
3. The first suggestions of fiber tubule formation are seen around the pericentriolar bodies at early prophase.
4. The secondary centriole, with no direct relation to the spindle fibers, is relatively free of the dense, amorphous substance.
5. The appearance of the primary centriole in late prophase, with spindle fibers becoming more clearly discernible as they emerge from tapering projections (Fig. 12), is highly suggestive that the amorphous substance takes part in their formation.

As the cell enters mitosis, the pericentriolar bodies seen at the stage of budding (Figs. 9, 10) give way to an amorphous substance which spreads out from the centriole. Fig. 8 shows this halo, and peripheral to it, suggestions of remnants of pericentriolar bodies. Possibly the amorphous substance previously has given origin to the satellite bodies and now is giving origin to the tubules of the spindle apparatus. The relation of the centriole to tubular structures may be more fundamental than their relation to a mitotic spindle, since similar tubules have been seen in relation to the centrioles (kinetosomes) in such specialized, nonmitotic cells as the sensory cells of the retina (12). Gall (16) has shown that the centrioles in the snail spermatocyte are capable of producing a large and variable number of kinetosomes, and in other situations appear to give origin to tubular astral fibers. In our material, as well as that of others, there is a general similarity between the centriolar tubules and spindle tubules, and de-Thé (10) has remarked that centriolar tubules might act as templates for microtubule formation. These

FIGURE 16 Thymocyte in early telophase. Interphase cell above shows a cytoplasmic bleb (arrow). × 13,000.

FIGURE 17 Equatorial region of a thymocyte in telophase. A ribosome-free band (*A*) beneath the plasma membrane is continuous with a similar, although vesiculated, band across the equator. Note the duplicated nuclear membrane (*B*) on the daughter nucleus. × 35,000.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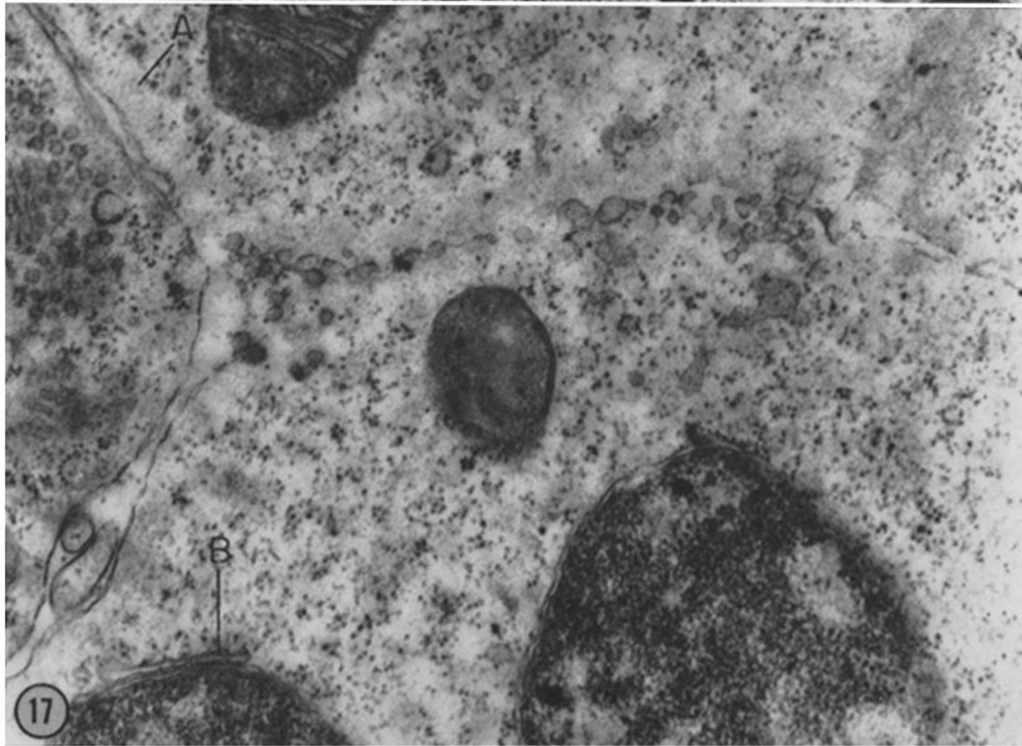
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17

considerations support a general concept, first advanced by de Harven and Bernhard (9), of centrioles capable of producing material which forms elements of new centrioles, a spindle apparatus, cilia, and perhaps other structures. It must be remembered, however, that plant cells also have tubular spindle fibers whose formation cannot be ascribed to organized centriolar structures (22).

Ribosome Bodies

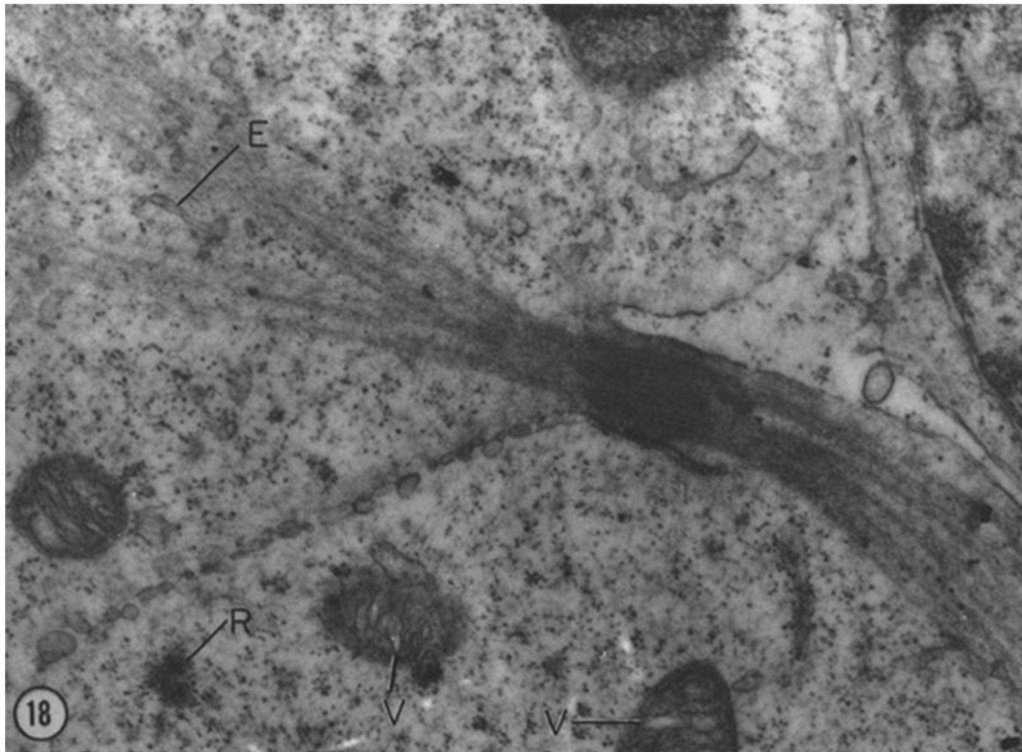
In resting cells and at early stages of mitosis, we find ribosomal aggregates of an order such that they might be considered polyribosomes (45). Beyond prophase, however, we find occasional masses of ribosomes which are too large and irregular to be so designated. They are often seen near the equatorial plate at telophase and resemble the ribosome bodies reported by Jones (20) in dividing erythroblasts. Ris and Kleinfeld (32) report that in certain *Lepidoptera* a process of chromatin elimination occurs in which basophilic bodies are shed by the chromosomes and move toward the equator. They showed that this material is largely RNA with some protein. Since ribosomes are said to be largely RNA with protein (29) there may be some analogy between these processes. Ris and Kleinfeld (32) speculate that the RNA movement may be related in some way to the stainable material eliminated in the formation of the midbody. The first appearance of density in the thymus midbody is a diffuse, nongranular material which never has the appearance of ribosomes. The ribosome bodies remain with the daughter cells and there is no indication that they are eliminated either with the midbody or in any other way.

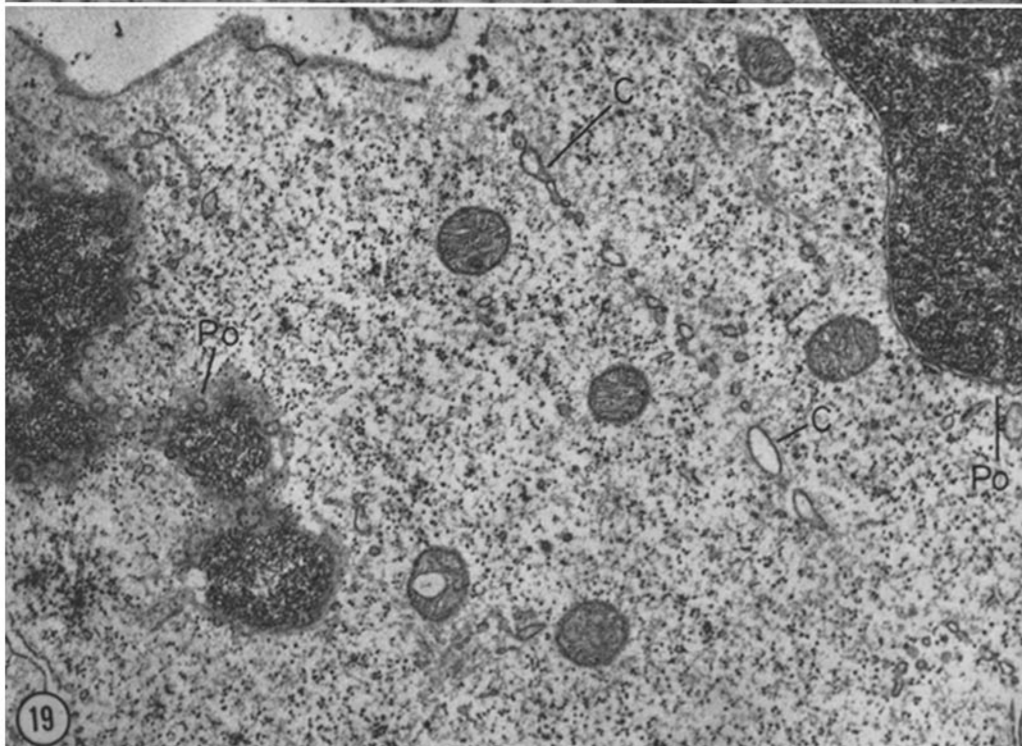
Cytokinesis

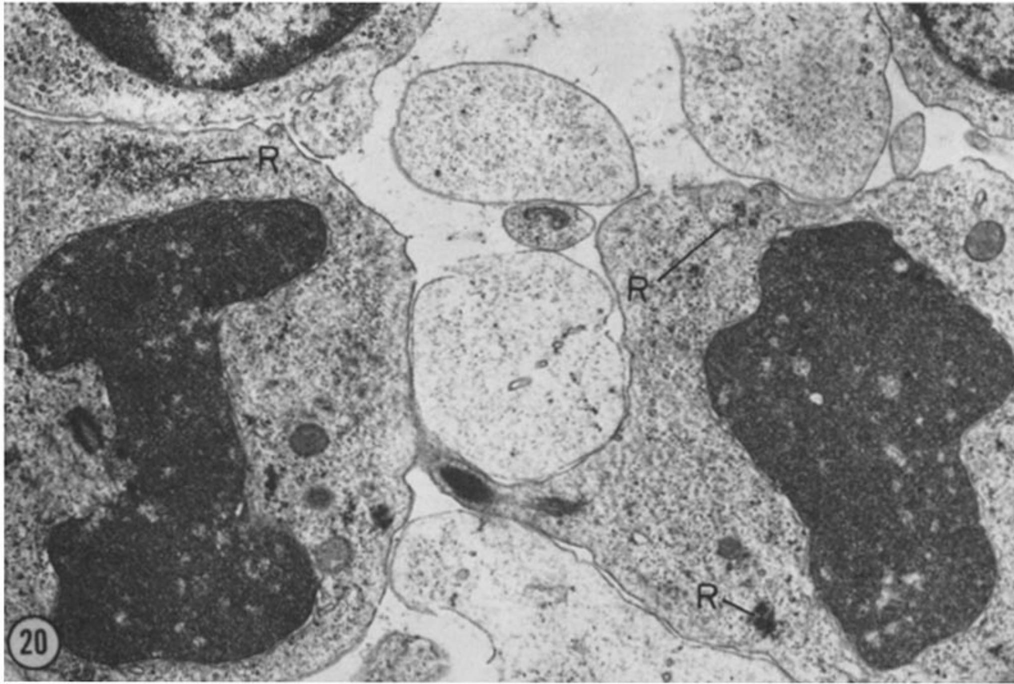
Swann and Mitchison (42) in their discussion of mechanisms of cleavage in animal cells point out that there must be an increase in surface area to encompass the two new cells, and suggest that this occurs either by membrane expansions or by growth of new membrane. The concept of membrane expansion is based on the analysis by Mitchison (26) of data relevant to the cell membrane which leads him to propose a structure consisting of a largely lipid "permeability layer", corresponding in many respects to the unit membrane of Robertson (34), but having, in addition, a much thicker (0.5 μ or more) underlying layer of protein molecules. The latter molecules are believed to be so oriented as to permit great expansion in area of the membrane if the molecular orientation is disrupted. We have commented previously (27) on the existence of a layer beneath the conventional plasma membrane of thymocytes which, although never so broad as 0.5 μ, in other respects might qualify as the oriented protein layer Mitchison postulates. This is an essentially amorphous zone with a viscosity apparently much higher than that of the cytoplasm in general, as indicated by the absence of ribosomes and by the fact that mitochondria seem unable to compress the material of this zone sufficiently to come into direct contact with the osmiophilic plasma membrane. In late anaphase in dividing thymocytes, a band of ribosome-free material forms a plate in the plane of the equator, which is traversed by spindle fibers. We have been struck by the similarity of the material in the plate to that in the zone beneath the plasma membrane, with which it is in direct continuity at the equator (Fig. 17). Vesicles form a row in the center of the plate, and presumably fuse to form the two new plasma membrane surfaces of the daughter cells. This mode of separation would seem to support a membrane growth hypothesis of cleavage, unless the appearance of material in the equatorial plane is a result of membrane expansion of the underlying protein layer only. The source of the vesicles cannot be determined, but remnants of the nu-

FIGURE 18 Region at equatorial plate of a thymocyte in telophase, showing well developed midbody. Note vacuolated crista membrane spaces (*V*) in mitochondria, ribosome body (*R*), and smooth-surfaced endoplasmic reticulum among the spindle fibers. (*E*). × 35,000.

FIGURE 19 Portion of equatorial plate of a thymocyte in telophase. Note enlargement and possible coalescence of vesicles at (*C*). Numerous pores are apparent (*Po*) in both oblique and transverse sections in the daughter nuclear envelopes. × 19,500.


E
R
V
V
18


C
Po
C
Po
19



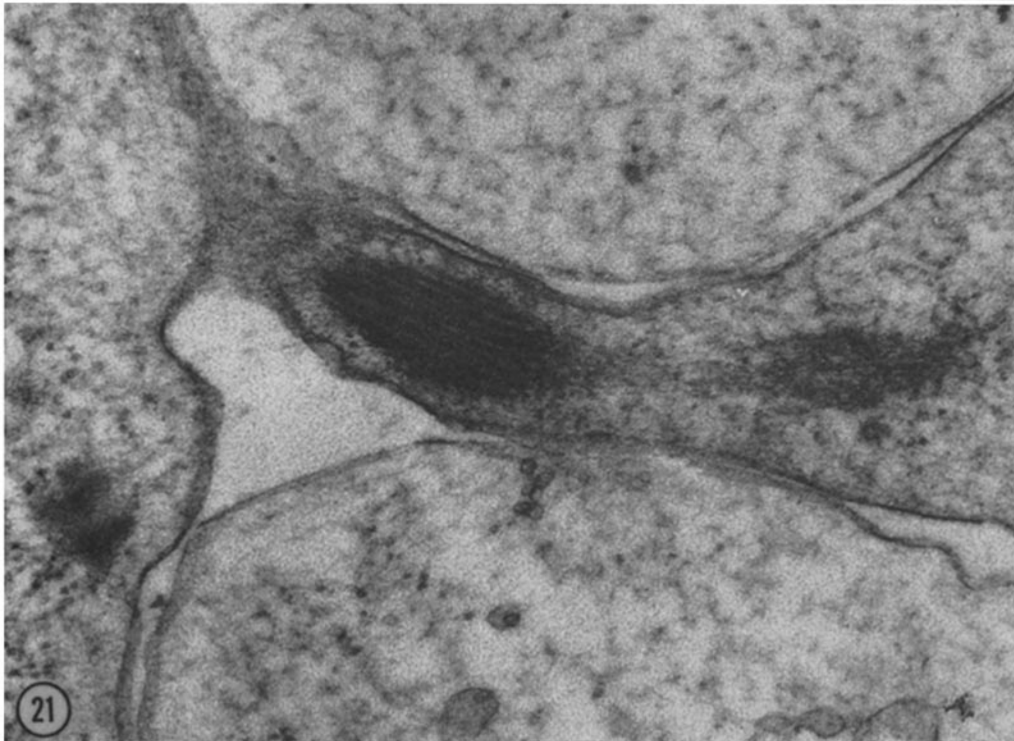


FIGURE 20 Thymocyte in late telophase showing midbody. Numerous ribosome bodies (*R*) are seen × 13,000.

FIGURE 21 Enlargement of the region of the midbody formation from Fig. 20. × 58,000.

clear envelope, or elements of the endoplasmic reticulum, insofar as these are distinguishable from each other, would be the most likely possibilities. The possibility must be considered that the vesicles represent a breakdown of a double membrane. Such a result has been postulated by Tormey (44) to be an artifact of OsO_4 fixation. If so, the sudden appearance of this membrane in this region has no explanation in our material.

The method of cell separation we see in thymocytes corresponds closely to that occurring in plant cells as described by Porter and Machado (30). Buck (7) has described a similar process in embryonic erythroblasts of the rat, but Robbins and Gonatas (33) did not see a vesiculating equatorial plate in the HeLa cells, nor are there cytoplasmic changes ahead of the advancing furrow in the cleavage of sea urchin eggs (49). One possible explanation of this difference might be that cells confined in a relatively solid tissue are subject to conditions similar to those in plant cells, which are confined within their cellulose compartments, and divide by a similar process, while cells in tissue culture might take advantage of greater freedom of movement to simplify the process. However, Dougherty (11) did not find vesiculating equatorial plates in dividing rat liver cells. Mitchison (26) has pointed out that the expanding membrane hypothesis can account for the increased surface area at cleavage in free-moving forms without the necessity of any new growth of membrane. Sea urchin blastomeres, where some freedom of movement must be lost, apparently show an intermediate type of cleavage, without a complete equatorial plate, but with some expansion inward from the equator of vesiculating material (48). However, in the dividing egg of *Artema salina* (14) a deep interblastomeric wall forms in the equatorial plane which appears to be a double plasma membrane, with no indication of a prior, broad band of amorphous material, or row of vesicles. Further details on formation of this plate in this species are needed. The nature of the broad band of material in the equatorial plane of mitotic cells, and of the band beneath the plasma membrane in resting cells, certainly should be investigated in detail.

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