THE ULTRASTRUCTURE OF SYNCHRONIZED HE La CELLS

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SUMMARY

Synchronous populations of mitotic HeLa cells were obtained by the controlled agitation method, and a detailed morphological study of the cells in all phases of the cell cycle was undertaken to correlate variations in cell structures to known coexisting biochemical events. Autoradiographic techniques using tritiated thymidine (3H-TdR) were used to detect S cells, and colcemid was added to some G2 samples to prevent the cells from going into the next cycle, thus preventing contamination with G1 cells.

The approximate duration (in hours) of the 4 phases were as follows: M = 14, G1 = 8-9, S = 7, G2 = 4, and the generation time (T) = 21 ± 2 h. Randomization of the cell populations became apparent in the G2 phase (contaminated with S and M cells) and was most likely a result of the genetic make-up of the individual (mixoploid) HeLa cells, nutritional factors (serum lots used), temperature shock, and handling effects. Polyribosomes shifted to monomeric ribosomes during late prophase, at which time nucleoli also break down. These changes are correlated with the drop in protein and RNA synthesis reported for mitotic mammalian cells. The Golgi apparatus persisted in a modified form throughout mitosis. The mid-body formed from the anaphase stem-body and may interfere with the separation of daughter cells. Our studies suggest that the mid-body goes to one of the daughter cells where remnants of this structure were seen in early G1 cells. Large numbers of polyribosomes and the presence of well-developed nucleoli (many attached to the nuclear envelope) characterized G1. These structures, which play a major role in protein and RNA synthesis, persist with slight variations throughout interphase. Dense fibrillar nuclear bodies were prominent in late G1 cells. Centrioles separate during G1, and replicate by orthogonal budding during the S phase. Reproducible labelling patterns which reflect the asynchronous multireplicon nature of DNA synthesis in mammalian cells were characteristic of the various stages of the S phase. Granular nuclear bodies, which were prominent in S and G2 cells, may correspond to the larger species of heterogeneous nuclear RNA found in HeLa cells. G2 cells were similar in appearance to S cells. As heterochromatin areas increased in late G2 and prophase, perichromatin granules (of unknown significance) became prominent. Mitochondria behaved as independent cell organelles throughout the cell cycle.

Hypertrophied RER, SER, and annulate lamellae, characterized the cytoplasm of colcemid-treated cells. The above changes are indicative of increased metabolic activity, and these structures may function in the production of colcemid-detoxifying enzymes in a manner analogous to that of drug-treated hepatocytes, such as those treated with phenobarbital.

INTRODUCTION

Although there is a voluminous literature concerning the biochemistry of synchronized mammalian cells (reviewed by Baserga, 1965, and Prescott, 1968), there has been no complete ultrastructural study of all 4 phases of the cell cycle (mitosis, G1, S, G2). Most ultrastructural reports dealt with cells in the easily identifiable stages
of mitosis and referred to all other cells as interphase cells. Structural changes in
different phase interphase mammalian cells were occasionally alluded to in the litera-
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ture. Robbins & Scharff (1966) noted that aside from changes in total mass, there is
very little visible change in the Golgi apparatus, lysosomes, endoplasmic reticulum,
mitochondria, or intranuclear structures in HeLa cells as they pass from one mitosis
to the next. Robbins, Jentzsch & Micali (1968), however, reported centriole and peri-
centriolar changes in different phases of the cell cycle. The 2 orthogonal centrioles
separate during $G_1$ and replicate in the $G_1-S$ transitional and $S$ period. Centriole
replication is completed by $G_2$. In $G_2$ marked pericentriolar changes occur, with each
centriole pair moving to opposite poles of the cell ultimately to reside in the two
daughter cells. Blondel & Tolmach (1965) noted a progressive decondensation of
chromatin as HeLa cells move from telophase to $G_1$. No morphological differences
between $G_1$ and $S$ nuclei were observed. Aside from these observations on HeLa cells,
there have been no other studies of variations in fine structure during the $G_1$, $S$, and
$G_2$ periods of the cell cycle in mammalian cells.

The following detailed light- and electron-microscopic study of the complete HeLa
$S_3$ cell cycle was undertaken in the hope of finding variations in cell structures and
possibly correlating them to known biochemical events taking place during each of
the 4 component phases. HeLa cells were synchronized by the controlled agitation
method (Terasima & Tolmach, 1963; Robbins & Marcus, 1964), which imposes
minimal chemical and physical stresses on the cells. The component phases were
identified by incorporation of tritiated thymidine ($^{3}$H-TdR—$G_1$ and $S$) and by direct
observation of mitotic stages. The spindle-inhibitor colcemid was used to obtain
populations of $G_2$ cells free from contamination with $G_1$ cells from the next cycle.

MATERIALS AND METHODS

Cell culture techniques

HeLa $S_3$ cells were grown in monolayers in $6.5 \times 11.0$ cm flat plastic flasks (Falcon Plastics,
California) using GIB (all media and sera were obtained from the Grand Island Biological Co.,
New York) minimum essential medium with Earle's salts supplemented with 10% calf serum.
The antibiotics used were: penicillin G (100 units/ml), streptomycin (0.05 mg/ml), and fungi-
zone (0.003 mg/ml). All cultures were maintained at 37 °C in a humidified incubator set for a
5 % CO$_2$ in air atmosphere.

Relatively pure (90 %) populations of metaphase cells were obtained by the controlled
agitation technique (Terasima & Tolmach, 1963) as modified by Kim & Perez (1965). Three-
day stock cultures were trypsinized (0.05 % isotonic trypsin) and approximately $8.0 \times 10^8$ cells
were transferred to each of 12 flasks. After the cells were cultured for 3 days, the regular medium
was removed and replaced with 40 ml of minimum essential medium with spinner salts (medium
minus calcium) supplemented with 10 % calf serum and antibiotics (a low calcium medium—
Robbins & Marcus, 1964). The cells were then incubated at 37 °C for 4–8 h; 2 h before use the
flasks were agitated and washed with low calcium medium to eliminate floating and dead cells.

In order to collect the mitotic cells from the random cultures, the flasks were stoppered
tightly and shaken two at a time (holding the arms in an extended position) to dislodge
the rounded, loosely attached metaphase cells. The resulting cell suspension from each of the 12
flasks was then poured into 12 50-ml plastic conical centrifuge tubes. Forty millilitres of fresh
low-calcium medium was immediately added to each of the culture flasks which were then re-
incubated for future collections. At the same time the suspended mitotic cells were centrifuged
at 800 rev/min for 3 min at room temperature. The cell pellets were resuspended in 1 ml of
regular medium and pooled. The number of mitotic cells collected at each shake varied from $4.4 \times 10^4$ to $1.8 \times 10^5$ cells/ml. The average number collected was $9.3 \times 10^4$ cells/ml. The 12-ml mitotic cell suspension was distributed as follows: 1 ml to 2 coverslip cultures; 1 ml for a cell count; and 9 ml (approximately $8.3 \times 10^4$ cells/dish) to an 8-cm plastic Petri dish. The 3 dishes were then placed in the incubator until the desired phase of the cell cycle was reached. Two drops of the cell suspension were mixed with acetic-orcein on a slide and checked microscopically. At this time 90% of the cells were in mitosis. The whole procedure was repeated at hourly intervals. Six experiments were carried out and 2-4 collections were made per experiment (Fig. 1, p. 356).

For thymidine incorporation studies, the medium was removed from the 8-cm Petri dish and 5 ml of regular medium containing $2.5 \mu$Ci/ml, 19 Ci/mmol, of $^3$H-TdR (thymidine methyl-$^3$H—Schwarz Bioresearch Inc., New York) was added to the cells; 15 min later the cells were rinsed twice in regular medium and then fixed for electron microscopy (see below).

### Table 1. Collection times (after synchronization) used to obtain cells in the different phases of the cell cycle

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time (h)</th>
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<tbody>
<tr>
<td>$M_1$, Early $G_1$</td>
<td>0.75, 19-22</td>
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<tr>
<td>Mid $G_1$</td>
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<tr>
<td>Late $G_1$, Early S</td>
<td>9</td>
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<tr>
<td>Mid S</td>
<td>14</td>
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<td>Late S, $G_2$</td>
<td>17-18</td>
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To prevent contamination of $G_2$ samples by $G_1$ cells of the next cycle, the medium was removed 4 (Expts. 14, 17) or 3 h (Expt. 18) before collection time, and medium containing 0.06 μg/ml (1.6 × 10^{-7} M) of colcemid (CIBA Pharmaceutical Co., New Jersey) was added—no colcemid was added to the $G_2$ cells from Expt. 12. At this dose not a single anaphase could be observed, while toxic effects appear minimal. Metaphase arrest was readily reversed by the addition of regular medium.

At the specified collection time for each phase of the cell cycle studied (Table 1), one coverslip was stained with May-Grünwald-Giemsa and the other by the Feulgen-light green method.

### Microscopy

At collection times (Table 1), the medium was gently poured off the 8-cm Petri dish culture and 5 ml of phosphate-buffered glutaraldehyde (1%)-acrolein (1%) fixative (Sandborn, Koen, McNabb & Moore, 1964) was poured over the cells. The fixative contained trace amounts of CaCl₂. The pH of the fixative was 7.27 and the osmolality was 393 mOsm/kg H₂O. The cells were fixed in situ for 10 min and gently scraped off the plastic with a rubber policeman. The cells were then pelleted and fixation was continued for 15 more min. The cell pellet was post-fixed for 1 h in 2% osmium tetroxide (Millonig, 1961). The pH was 7.23 and the osmolality was adjusted to 366 mOsm/kg H₂O with glucose. In one experiment (12), the cells were trypsinized off the plastic, pelleted, and then fixed. After dehydration in ethanol, the pellets were embedded in a mixture of Maraglas and Dow Epoxy Resin 732 (Erlandson, 1964). Thin sections were stained with uranyl acetate (Watson, 1958), followed by lead citrate (Reynolds, 1963), and examined in a Siemens Elmiskop 1 electron microscope. Thick sections (0.5-1.0 μm) of epoxy-embedded tissue were stained with toluidine blue by the method of Björkman (1962). Micrographs were taken with a Leitz Labolux microscope equipped with planapochromatic objectives. Synchronized cultures were also placed in a Sykes-Moore chamber and observed using phase optics. The microscope stage was maintained at 37°C and was supplied with a 5% CO₂-air mixture.
 Autoradiography

For light- and electron-microscope autoradiography the technique of Caro & Van Tubergen (1962) was used, with minor modifications (Ilford L 4 emulsion was used, and the specimens were developed in Kodak D 19).

The mitotic indices (the number of cells in mitosis per 100 cells), and the labelling indices (the number of labelled cells per 100 scored), were calculated from light-microscopic preparations. For each specimen a total of 1000 cells was counted.

RESULTS

Cell cycle analysis

In random confluent cultures of HeLa cells, approximately 4.7% of the cells were in the various stages of mitosis (MI = 4.7). Cells and nuclei varied in size and shape and some abnormal mitotic figures, mainly multipolar metaphases, were present. This is a characteristic feature of mixoploid HeLa cell lines which were originally obtained from cancerous tissue.

Approximately 90% of the cells obtained by the mechanical selection technique were found to be in mitosis—mainly metaphase. Since 45 min elapsed between the collection of mitotic cells and the fixation of the cell pellet for electron microscopy, very few prophase cells were seen, and many of the metaphase cells progressed to telophase, or just past mitosis into early G1 (Fig. 2). Therefore, the 'mitosis' preparations consisted mainly of metaphase, telophase, and early G1 cells. Mitotic cells (from prophase to telophase) were also examined at the end of one cell cycle (19 and
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21 h) when approximately 20% of the cells were again dividing. Phase-contrast observations revealed that anaphase was very rapid (1–2 min) and metaphase lasted from 20 to 40 min. Both prophase and telophase lasted approximately 20 min.

From Fig. 1 it is evident that the number of mitotic cells dropped to about 1% of the total cell population by 4 h and did not begin to increase again until about 15 h after synchronization. The percentage of cells synthesizing DNA rose sharply at 9 h and approximately 85% of the cells were in S phase by 14 h. The number of labelled S cells dropped between 14 and 17 h.

Phase-contrast observations indicated that the duration of mitosis (tm) varied from cell to cell, ranging between 40 and 90 min for the limited number of cells followed. Some of the cells seemed to remain in metaphase indefinitely. The duration of mitosis was also calculated from the population doubling time (T), and the MI obtained from asynchronous cultures by use of the equation of Stanners & Till (1960):

\[ tm = M \frac{T}{0.693} \]

where \( M = MI/100 \). From Fig. 1 it is apparent that the cell population had doubled by approximately 21 h (obtained by assuming \( tm = 1 \) h and adding the mitotic indices between 14 and 21 h, which totals to 100%). Phase-contrast analysis (time-lapse movies) also indicated that 21 ± 2 h elapsed between the midpoint of a successive mitosis in the same cell; therefore, \( T = 21 \) h. Since the MI of a random population of HeLa cells equals 4.7, by substitution of the above values the average \( tm \) comes out to 1.4 h.

The duration of the \( G_1 \) phase (\( t_{G_1} \)) is approximately equal to the time between cell division and the appearance of labelled cells (Watanabe & Okada, 1967). Fig. 1 shows that 8–9 h elapsed before the percentage of labelled cells rose abruptly; therefore, \( t_{G_1} = 8–9 \) h.

The duration of the \( S \) phase (\( t_s \)) corresponds approximately to the interval between the two 50% points on the ascending and descending limbs of the labelling-index (LI) curve of Fig. 1 (Sisken & Morasca, 1965). These points correspond to 11 and 18 h and therefore \( t_s = 7 \) h.

The probable duration of the \( G_2 \) phase (\( t_{G_2} \)) was estimated at 4 h by subtracting the sum of \( t_{G_1}, t_s, \) and \( tm \) from the generation time (T). Errors in the determination of the duration of the other phases further reduce the accuracy of the \( t_{G_2} \) estimation. Randomization of the cell population accounts for the spread of values, especially in the \( G_2 \) region (see Discussion). The above parameters are in good agreement with those of other investigators (Terasima & Tolmach, 1963; Puck, 1964; and J.H. Kim, personal communication of 1968).

Structural variations during the cell cycle

Mitosis. Since Robbins & Gonatas (1964a) and Robbins & Jentzsch (1969) reported on the ultrastructure of mitotic HeLa cells, only supplemental observations made in this study, notably the events of telophase and the transition to interphase (\( G_1 \)), will be described in some detail.
Prophase was characterized by polar localization of the centrioles, the presence of a large number of spindle tubules in the pericentriolar osmiophilic zone (Fig. 3), rounding of the cell, the appearance of chromosomes, marked irregularity of the nuclear envelope, and dispersal of nucleolar constituents. In late prophase the nuclear envelope fragmented into segments which probably persisted throughout mitosis. Small clusters of lysosomes were present in the cytoplasm of many of the prophase cells. Except for the centrioles, no significant changes in the cytoplasmic organelles were evident.

During late prophase and early metaphase the chromosomes began their migration toward the cell equator. The chromosomes became aligned at the equator, forming the metaphase plate (Fig. 4). Mitochondria, clusters of lysosomes, short segments of rough endoplasmic reticulum (RER), and arrays of nuclear envelope remnants, were excluded from the spindle (centrioles, spindle tubules, and chromosomes) region of the cell (Fig. 4). The diplosomes (at each pole of the spindle) were typically encased by a granular osmiophilic halo, and numerous spindle tubules (20 nm in diameter) were seen radiating in all directions from this region (Fig. 5). Typical kinetochores were also noted in favourable sections. No nucleoli or nucleolar remnants were seen until late telophase. Individual ribosomes were scattered throughout the entire metaphase cell and very few polysomes were evident. Ribosomes predominated from late prophase to early telophase. At variance with Robbins & Gonatas (1964a), small vesicles and lamellar elements, which may represent Golgi structures, were occasionally noted (Fig. 6).

Separation of sister chromatids, now called daughter chromosomes, marked the beginning of anaphase. As anaphase progressed, the chromosomes moved toward the poles and soon began to fuse into electron-dense masses. The mitochondria and lysosomes congregated at the equatorial area between the parting chromosome masses. Elongated cisternae (early nuclear envelope formation) enclosed the fused chromosome masses on the sides facing the poles of the cell (Fig. 7). Concomitant with early chromatid separation, small clusters of spindle tubules were apparent in the interzonal region. As many as 5 of these structures or stem-bodies (Krishan & Buck, 1965) — the 'Stemmkörper' of Belar (1927) — were observed in some anaphase cells. An electron-dense, finely granular material adhered to the clusters of tubules at the centre of the spindle (Fig. 7, inset). Lagging chromosomes were also evident in some anaphase and early telophase cells. By late anaphase, all the chromosomes had fused into one dense mass already enveloped by an almost complete nuclear envelope. The fine structure of the diplosome remained unchanged.

The end of the polar migration of the daughter chromosomes marked the beginning of telophase. Cytokinesis began at about the same time by symmetrical invagination of the plasma membrane in the equatorial region. Extensive blebbing of ectoplasm in the polar and furrow regions was evident in both living and fixed telophase cells. During this period nuclear reconstruction was essentially completed and chromatin had a decreased electron density. Most of the mitochondria and lysosomes were located in the furrow region of the cell, and occasional lipid droplets were seen (Fig. 8). The advancing furrow appears to push the stem-bodies together with the resultant forma-
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tion of a mid-body in the narrowing intercellular bridge (Fig. 9). By this time most of
the mitochondria were distributed in more or less equal proportions between the two
daughter cells. Some organelles, however, could still be seen in the intercellular bridge
(Fig. 9). Lysosomes were scattered throughout the daughter cells, and polysomes were
again prevalent in the cytoplasm.

By late telophase, chromatin decondensation continued and newly formed nucleoli
(usually attached to the nuclear envelope) were evident in the daughter cell nuclei.
Also at this time a dense mid-body with spindle tubules radiating from both sides
occupied most of the intercellular bridge (Fig. 10). A small Golgi apparatus, some-
times centred around a centriole, was prominent in the nuclear hof region of the newly
formed bean-shaped nuclei (Fig. 10).

Spindle tubules (attached to the mid-body) became disarrayed and eventually
were no longer present in one of the 2 daughter cells as illustrated by Figs. 11–13. The
mid-body became separated from one daughter cell while remaining an integral part
of the other, thereby introducing a factor of asymmetry in the last phase of mitosis
(Figs. 13, 14). The completion of cell separation marked the beginning of early
G1.

**Interphase.** During early G1 most of the mid-body and its associated spindle tubules
progressively disappear; only remnants of these structures were occasionally seen in
daughter cells (Fig. 15). No mid-body remnants were seen in cells from mid G1 on.
By mid G1 (4 h) many of the spherical daughter cells still remained close together and
appeared as doublets in the cell preparations. Extensive cytoplasmic blebbing of the
ectoplasm of the rounded cells was evident throughout the G1 phase.

Early G1 cells possessed a characteristic bean-shaped nucleus with a prominent
nuclear hof (Fig. 16). A large Golgi apparatus, composed of vesicles of various sizes
and multilayered arrays of flat cisternae, some with bulbous ends, occupied this
region of the cell (Fig. 17). The Golgi apparatus remained morphologically unchanged
throughout the rest of G1, and the S and G2 phases (Fig. 23). Clusters of lysosomes
were usually present in the Golgi area of the cells, and these aggregates were most
prominent in G1 (except for the late G1 phase) and G2 cells (Fig. 17). A pair of centrioles
was seen in the Golgi area of favourably sectioned cells (Fig. 16). While the centro-
sphere was poorly developed in mid G1 and early S cells, centriole satellites (70–90 nm
in diameter) were very prominent in mid S cells, and some of these structures made
contact with the walls of the centriole (Fig. 18 inset, and Fig. 19). Orthogonal pro-
centriole formation, with a short bud protruding from each member of the pair, was
evident in favourable sections (Fig. 18). Microtubules, which could be seen scattered
about the cytoplasm of interphase cells, were sometimes seen in the centrosphere, with
their polar end in apparent continuity with the centriole satellites (Fig. 19). Two pairs
of mature centrioles were present in the centrosphere by late G2 or early prophase
(Fig. 20).

Some small patches of condensed chromatin were still evident in many of the G1
nuclei which had rounded up by mid G1. These heterochromatin areas progressively
decreased in number up until the early S phase, and then progressively increased in
number right up to the mitotic phase. Dense perichromatin granules (Watson, 1962),
53–57 nm in diameter, were evident in regions of nucleolus-associated chromatin and heterochromatin (Fig. 21). Large masses of heterochromatin probably indicated chromosome condensation during the later stages of G₂.

The 4-h cultures consisted mainly of G₁ cells (95–98%), a few persisting mitotic cells, and rare (1%) labelled cells. Some binucleated cells were seen. Seventeen per cent of the cells in the 4-h sample (Expt. 18) contained from 6 to 20 silver grains in their nuclei (Fig. 22). The incorporation of ³H-TdR into the nuclear DNA marked the beginning of the S phase. Most of the grains were located over regions of dispersed chromatin (euchromatin), and only rarely over the nucleolus and near the nuclear envelope (Fig. 22). By mid S (14 h) 80–85% of the cells were heavily labelled (average of 30 grains per nucleus). In most cells the grains were distributed throughout the whole nucleus (nucleolus, euchromatin, and along the nuclear envelope) in contrast to the early S cells (euchromatin only)—Fig. 23. The labelling pattern was similar in cells fixed both after trypsinization (Expt. 12) and in situ. Very few mitotic cells (1–2%) were still present, and the unlabelled interphase cells could be in either G₁ or G₂. Most of the cells had flattened out by this time and microvilli were seen only on the part of cell surfaces not attached to the glass. The late S and G₂ (17.5 and 18 h) samples were composed of a mixed population of cells because of progressive randomization of the cell populations. The samples consisted of 15% mitotic cells (in all stages of mitosis except where treated with colcemid), 34% labelled S cells, and the remaining 50% consisted of G₂ cells (mixed with some early G₁ cells from the next cycle only in Expt. 12 where no colcemid was added)—Fig. 24. Most of the S cells were labelled predominantly around the nucleolus and along the nuclear envelope (late S pattern) irrespective of whether the cells were treated with colcemid or not (Fig. 25).

Small nucleoli (up to 4 per cell), many of which were in close contact with the nuclear envelope, were evident in the early G₁ nuclei (Fig. 16). By mid G₁ (4 h) the nucleoli had increased in size, and many of them were still attached to the nuclear envelope. By late G₁ (9 h), however, many of the large nucleoli were no longer in contact with the nuclear envelope. Nucleoli remained relatively unchanged for the rest of interphase; they were well differentiated into granular and fibrillar components. Granules, ranging in size from 15 to 18 nm, made up the bulk of the structure (Fig. 26). Prominent circular profiles of tightly packed fine fibrils (fibrillar component) and occasional 25-nm granules permeated the mass of the nucleolus (Fig. 26).

Dense (24–46 nm) nuclear bodies composed of tightly packed intertwined fibrils ranging from 3 to 10 nm in diameter were seen in many of the HeLa cell nuclei from mid G₁ throughout the rest of interphase (Fig. 26). These structures were most prominent in late G₁ nuclei (Fig. 27). Another type of nuclear body, appearing more granular and ranging in size from 570 to 810 nm in diameter, was made up of rather large (39–60 nm) dense granules (Fig. 28); these were occasionally seen in G₁ cells. Granular nuclear bodies were most prominent in S and G₂ cells. Both types of nuclear bodies did not incorporate ³H-TdR (Fig. 28).

By mid G₁, short segments of RER and numerous polysomes were prevalent in the cytoplasm. Polysomes were most numerous in the cytoplasmic extensions of the flattened cells and were composed of 4–5 ribosomal units (Fig. 29). Helical configurations, up to
Elements of SER were not evident except in colcemid-treated cells (see below).

Mitochondria of various sizes and shapes were seen in all interphase cells. Mitochondria with a dense matrix usually contained prominent mitochondrial ribosomes (Fig. 29). Many of these organelles contained electron-lucent regions with small clusters of fine fibrils which may represent the mitochondrial DNA (mDNA). These electron-lucent regions were most prominent in the $G_2$ cell mitochondria (Fig. 21).

Typical annulate lamellae (see colcemid section) were seen in a juxtanuclear position in $G_1$ (rather rare), $S$, and $G_2$ cells (Fig. 23). A dense fibrillar-granular material was concentrated in the annuli of the lamellae (Figs. 30 and 35 inset). In a number of the $S$ cells examined, the lamellae were attached end-to-end to parallel rows of RER (Fig. 30).

Colcemid-treated cells

Synchronized cell populations fixed and examined at 17.5 and 18 h in Expts. 14, 17 and 18 (Fig. 1) were exposed to 0.06 μg/ml of colcemid for 4 h (Expts. 14, 17), or 3 h (Expt. 18). In all the above preparations, typical C-metaphases were seen in varying numbers (Fig. 31). No anaphases or telophases were noted. These samples therefore were not contaminated with $G_1$ cells from the next cycle.

The nuclei of colcemid-treated cells were of a similar morphology to those of untreated cells discussed in previous paragraphs, containing both types of nuclear bodies, well-developed nucleoli (many still marginated), and prominent patches of heterochromatin with associated perichromatin granules (Fig. 32).

Colcemid exerted its most pronounced effects on a number of cytoplasmic structures. The Golgi complexes of the cells were hypoplastic and small stacks of Golgi lamellae and vesicles were scattered about the cytoplasm (Fig. 33). Fragmentation of the Golgi apparatus was previously noted in HeLa cells treated with 0.1-1.0 μg/ml of colchicine or vinblastine sulphate (Robbins & Gonatas, 1964b). Lysosomes were present in treated cells. Centrioles and centriole satellites, which were located in the small Golgi zone, were unaffected by the drug, while microtubules had almost completely disappeared (Fig. 34). Polysomes and mitochondria also were apparently not altered (Figs. 33, 35, 36).

The most prominent changes which occurred in the cytoplasm of treated $S$ and $G_2$ cells consisted of hypertrophy (or increased production) of RER and annulate lamellae, and the appearance of large whorls and linear arrays of smooth endoplasmic reticulum (SER). Parallel cisternae of RER were frequently observed to be in direct continuity with annulate lamellae, in the same fashion as in $S$ cells (Fig. 35). In some cases, the above structures were also continuous with SER (Fig. 35). The SER was composed of isolated elliptical and circular profiles or short segments of branching tubules arranged in a loose-meshed tortuous fashion (Fig. 36). Glycogen particles were also present within the interstices of the complex of tubular smooth membranes (Fig. 36). Fig. 36 shows a helical polysome in the centre of an SER whorl. The SER structures differed from annulate lamellae in that no granular-fibrillar material was present in attenuated regions of the cisternae. Some scattered glycogen particles were
located between the membranes of the annulate lamellae. SER profiles were very rare in untreated cells.

Colcemid-arrested mitotic cells (C-metaphases) displayed the same changes described above. There was no evidence of an organized spindle, and chromosomes were scattered randomly about the cytoplasm.

The results are summarized in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Cell constituent</th>
<th>PMAT</th>
<th>G1</th>
<th>G1 Mid</th>
<th>G1 Late</th>
<th>S</th>
<th>G2</th>
<th>G2 Mid</th>
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<td>1. Continuous nuclear envelope</td>
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<td>2. Nucleolus-nuclear envelope</td>
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<td>3. Nucleolus</td>
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<td>4. Granular nuclear body</td>
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<td>5. Fibrillar nuclear body</td>
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<td>9. Centriole satellites</td>
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**Key:**
- Present and normal
- Prominent
- Abnormal
- Absent (rare)
- Nucleolus in contact with nuclear envelope.
- † Refers to chromosomes during mitosis.
- ‡ Annulate lamellae in direct continuity with RER.

### DISCUSSION

From Fig. 1 it is obvious that the cell population desynchronized progressively, resulting in G2-phase contamination with mitotic (about 20%) and late S (about 30%) cells. In Expt. 12, where no colcemid was added, second-generation G1 cells were also present in the G2 samples. The cell population doubled over a period of 6 h, whereas if the cells still had retained a very high degree of synchrony, the population doubling time should have been approximately 1 h. Similar randomization was noted in HeLa
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cells by other investigators (Froese, 1964; Hsu, 1960; Kim & Perez, 1965; Scharff & Robbins, 1965; Terasima & Tolmach, 1963), and was most likely due to 4 main factors:
(1) the cell populations were not 100% synchronized to start with; (2) variations in the genetic make-up of the individual cells; (3) the nutritional environment (the different lots of serum used); and (4) the low temperature shock during the synchronization procedure which apparently can slow down some key metabolic processes during M and G₁ (Rao & Engelberg, 1968; Shapiro & Lubennikova, 1968; Watanabe & Okada, 1967). The 'shock' effects of centrifugation and pipetting should also be considered.

The majority of cells dislodged by the controlled agitation technique were in metaphase. Since there was a delay of approximately 45 min before fixation for electron microscopy, many of the cells passed through anaphase into telophase, while others progressed into the early G₁ period. This cell population provided an excellent opportunity to study the formation and fate of the mid-body in HeLa cells.

The earliest sign of mid-body formation was the aggregation of interzonal spindle tubules into small bundles during early anaphase. Small dense granules were clustered near the tubule walls midway between the two chromosome masses. Krishan & Buck (1965) referred to this structure as the stem-body because it was thought to correspond to the ‘Stemkörper’ of Belar. A similar granular material collected around phragmoplast microtubules in the African blood lily Haemanthus katherinae Baker (Hepler & Jackson, 1968). The early mid-body (stem-body) closely resembled the developing plant phragmoplast at this stage of formation. The advancing furrow gradually pushed these structures together resulting in the formation of a mid-body, usually in the central region of the intercellular bridge. In early telophase the bridge forms a possible pathway for the movement of cellular constituents between the two daughter cells. Cinematographic studies by Abramson & Byers (1966) demonstrated that complete separation is not achieved in HeLa cells until about 2–5 h after mid-body formation. Our studies indicated that scission occurred rapidly (about 30 min) in most telophase cells, but the daughter cells usually remain close together throughout most of G₁, forming cell doublets. Thus, the completion of cleavage appeared temporarily inhibited by the persisting mid-body. Prolongation of abscission, permitting the possible passage of materials between the connected cells, is especially prominent in testicular germinal cells (Fawcett, 1961). No mid-bodies are present in these bridges. The spindle tubule elements are composed of protein(s) (Inoué & Sato, 1967), while the chemical composition of the granular constituent of the mid-body is not known.

One of the intriguing problems related to late telophase is the fate of the mid-body. Robbins & Gonatas (1964a) theorized that the HeLa cell mid-body was cast off from the cell by a pincer mechanism from shallow plasma membrane invaginations at the extremities of the intercellular bridge. Jones (1969) showed that mid-bodies were eliminated from mitotic rat erythroblasts by a similar mechanism. Using electron-microscopic and cinematographic techniques, Byers & Abramson (1968) observed that the bridge elongates and eventually ruptures and goes to one daughter cell after the spindle tubules are withdrawn from this cell. Buck & Tisdale (1962) claimed that in many cell lines the bridge snaps and the mid-body is retained by one of the daughter
cells. Other investigators have suggested (but not illustrated) that the mid-body is discarded, as for example in the cells of the chick embryo (Allenspach & Roth, 1967), and in rat thymic lymphocytes (Murray, Murray & Pizzo, 1965). Our data strongly suggest that the spindle tubules in one of the daughter cells become disorganized and disappear; this is followed by rupture of the bridge. The mid-body remnant (Fig. 15) is retained by one of the daughter cells and the structure eventually breaks up and disappears within the cytoplasm. No remnants were seen in the 4-h $G_1$ preparation.

The nucleolus disappeared in prophase and rapidly reappeared in the newly formed nucleus in late telophase. As the cells progressed through $G_1$ the nucleoli increased in both size and complexity. Increased numbers of hypertrophied nucleoli are characteristic of cancer and other rapidly growing cells (Bernhard, 1966; Bernhard & Granboulan, 1963; Busch, Byvoet & Smetana, 1963; Vincent & Miller, 1966). This rapid nucleolar development correlates with the concomitant increase in RNA and protein synthesis which occurs throughout $G_1$ (Kim & Perez, 1965; Scharff & Robbins, 1965; Terasima & Tolmach, 1963). The HeLa cell nucleoli were at their largest size in mid $G_1$ where nucleolar RNA synthesis was increasing at a maximal rate.

Many of the hypertrophied nucleoli were attached to the nuclear envelope throughout interphase, especially during $G_1$. Marginated hypertrophied nucleoli are found in HeLa (Journey & Goldstein, 1961) and in many other types of cancer cells (Bernhard, 1966; Bernhard & Granboulan, 1963; Rubin, Sutton & Zimmerman, 1968), and in cells of rapidly growing tissues, such as embryonic hepatocytes (Dvôrák, 1964), haemopoietic cells in the foetal liver (Zamboni, 1965), pilocarpine-stimulated mouse pancreatic acinar cells (Bannasch & Thoenes, 1965), and regenerating hepatocytes after subtotal hepatectomy (Stenger & Confer, 1966). Peripheral displacement has also been observed in a wide variety of pathological conditions (Blackburn & Miller, 1968; Martinez-Palomo, Le Buis & Bernhard, 1967; Shinozuka, Verney & Sidransky, 1968). Localization of nucleoli at the nuclear envelope probably facilitates the movement of ribosome precursors into the cytoplasm. Our ultrastructural studies, along with those of other investigators (Shinozuka et al. 1968), failed to demonstrate conclusively the passage of material through nuclear pores in the area of nucleolar attachment.

Two types of nuclear bodies were present in the HeLa cell nuclei; one fibrillar, and the other granular. Nuclear bodies, first described by Weber & Frommes (1963), vary widely in size and configuration and are found in both normal and pathological tissues. They are especially prominent in the latter (Bouteille, Kalifat & Delarue, 1967; Popoff & Stewart, 1968; Zu Rhein & Chou, 1968). Bouteille et al. (1967) concluded that nuclear bodies are related to cellular hyperactivity, the cause of which may be physiological, hormonal, drug-induced, viral, or tumoural.

The dense fibrillar type of nuclear body was most prominent in late $G_1$ cells, but was also seen in late $S$ and $G_4$ cells. These structures may represent highly condensed heterochromatin that corresponds to the chromocentres of Feulgen-stained nuclei (Fell & Hughes, 1949). Feulgen-positive chromocentres were fairly prevalent in late $G_1$, $S$ and $G_4$ preparations. It is also possible that these structures are altered fragments of nucleolar material as suggested for similar structures in other cell types (Brinkley,
infrastructure of synchronized HeLa cells’ (1965; Popoff & Stewart, 1968). The adenosine analogue aminonucleoside produced fragmentation and clumping of the fibrillar component of HeLa cell nucleoli resulting in a nuclear inclusion identical in appearance to the fibrillar nuclear body (Lewin & Moscorello, 1968). This suggests that this structure could originate from nucleolar-associated DNA.

The granular type of nuclear body was especially prominent in S and G2 cells. The dense granules range in size from 39 to 60 nm. This structure appears identical to the accessory body of Cajal seen in neuronal nuclei (Hardin, Spicer & Greene, 1969), and the coiled body of mammalian cell nuclei (Monneron & Bernhard, 1969). The chemical composition of similar-looking nuclear bodies has not been determined (Zu Rhein & Chou, 1968). The accessory body of Cajal is derived from a paranucleolar structure, which is itself formed from the dense component of the nucleolus. Hardin et al. (1969) speculated that this structure may transport rRNA from the nucleolus to the nucleoplasm. No evidence for the hypothetical nucleolar origin of these structures was found in the present investigation. The granules may be collections of the larger types of polydispersed heterogeneous nuclear RNA’s found in HeLa cells. These RNA’s have a base composition similar to nuclear DNA, turn over rapidly in the nucleus, are unaffected by actinomycin D, and are not associated with the nucleolus (Houssais & Attardi, 1966; Soeiro, Vaughan, Warner & Darnell, 1968). Sedimentation velocities up to 5000 s have been reported (Penman, Vesco & Penman, 1968). The function of these large heterogeneous RNA’s is not known. Digestion and extraction studies by Monneron & Bernhard (1969) showed that a similar structure—the coiled body—contains nucleoproteins and further suggests that the polymorphism of nuclear components may correspond to the molecular heterogeneity of nuclear RNA.

Synthesis of both RNA and protein is continuous throughout interphase. The granular components of the nucleolus probably correspond to the different ribosomal precursors synthesized and stored within this structure (Bernhard, 1966; Knight & Darnell, 1967; Penman, Smith & Holtzman, 1966; Perry, 1964; Vaughan, Warner & Darnell, 1967).

Large perichromatin granules (53–57 nm) became increasingly prominent at the periphery of nucleoli and heterochromatin from late S up to early prophase. Watson (1962) noted an affinity for indium and uranyl salts and suggested that perichromatin granules are composed of nucleic acids. More recent reports indicate that these structures are found more frequently in tumour cells, are most likely made of nucleoprotein, and may be involved in the transfer of nuclear information from the chromosomes towards the cytoplasm (Bernhard & Granboulan, 1963; Monneron & Bernhard, 1969; Zu Rhein & Chou, 1968). The reason for their prominence in G2 and early prophase is not known, but the heterochromatin areas with which they are usually associated steadily increase during this period.

Studies of 3H-TdR incorporation into nuclear DNA during various periods of S revealed an interesting pattern of DNA synthesis. DNA synthesis began in euchromatic areas during the early S period (8 h), and progressively increased until grains were found throughout the nucleus at peak DNA synthesis (mid S—14 h). During late S (17.5–18 h), 3H-TdR incorporation was most evident close to the nuclear envelope
and within the nucleolus (nucleolus-associated chromatin). The short pulses used in these experiments permit us to rule out a progressive margination of the DNA synthesized in the euchromatic regions. This labelling pattern was reproducible, and is in good agreement with the findings of other investigators that DNA synthesis in mammalian cells is asynchronous (German, 1964; Moorhead & Defendi, 1963; Stubblefield & Mueller, 1962; Taylor, 1960; Tokuyasu, Madden & Zeldis, 1968), and that chromosomes are multireplicons (Cairns, 1966; Prescott, 1968). DNA synthesis in HeLa cells is a reproducible localized phenomenon and the chromosomes are similar to those of normal human cells although the total number varies (Stubblefield & Mueller, 1962).

Our findings confirm those of Mueller, Kajiwara, Stubblefield & Rueckert (1962) which demonstrated that the synthesis of DNA began at a slow rate and then accelerated as more and more inactive loci were progressively converted to a competent state for DNA synthesis. Maisel & King (1968) found that nucleolar \(^3\)H-TdR incorporation began later than non-nucleolar incorporation during early S. Kasten & Strasser (1966), however, observed that the nucleolar DNA (in CMP human epithelial adenocarcinoma) was the trigger site for DNA synthesis. These cells were synchronized by double-blockage with excess TdR, and can hardly be considered to be in a natural state. Heteropoloid human amnion cells synchronized with excess thymidine and amethopterin, incorporated \(^3\)H-TdR (10-min pulse after release—therefore early S) at the periphery of the nucleus and within the nucleolus (Comings & Kakefuda, 1968). These authors suggested that DNA synthesis in human cells may be initiated at the nuclear envelope in a manner analogous to the bacterial mesosome-replicator system. This method of synchronization induces a state of unbalanced growth, which could lead to morphological changes as well. Blondel (1968) found that the pattern of localization of \(^3\)H-TdR was essentially the same throughout the S phase of mitotically harvested KB cells. But the peripheral region of the nucleus was found to be more active in the latter part of S, as we have noted. Our experiments indicated that the use of trypsin to dislodge cells from the plastic substrate produces areas of clumped chromatin and generalized coarse nuclear fixation as reported by Blondel (1967), although without apparent effect on the nuclear labelling patterns.

The chromosome site for the initiation of DNA synthesis in human cells remains an enigma. Nuclear DNA synthesis was followed throughout the entire S period in the present experiment using ‘undamaged’ synchronized cells obtained by the selective detachment method. The plated-out cells were fixed \textit{in situ} to ensure minimal displacement of nuclear constituents. The resolution obtained from the \(\beta\)-emitter tritium is in the order of 0.1 \(\mu\)m (Caro, 1966), and colcemid-treated cells had an unaltered nuclear fine structure. All of the above factors, including reproducibility of labelling patterns, indicate that our results illustrated the normal pattern of DNA synthesis in HeLa cells.

The silver grains were localized predominantly over the dispersed (euchromatin) areas of the nucleus which are thought to be active in DNA synthesis, as opposed to the tightly coiled heterochromatin areas (Hay & Revel, 1963). No grains were ever found over the nuclear bodies. The above findings are in agreement with the electron-
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microscopic autoradiographic investigations of others (Blondel, 1968; Littau, Allfrey, Frenster & Mirsky, 1964; Tokuyasu et al. 1968).

Annulate lamellae represent a poorly understood class of cytoplasmic membranes. These structures are prominent in rapidly proliferating embryonic and neoplastic cells and in oocytes, and they are most likely a specialized form of endoplasmic reticulum derived from the nuclear envelope (Everingham, 1968; Kessel, 1968; Merkow, Slifkin, Pardo & Romano, 1968). Annulate lamellae are also found in normal adult human somatic tissues (Gross, 1966), although they are relatively rare in these cells. In the present study the lamellae first appeared in mid G₁ (4 h), and were especially prominent in S and G₂ cells, paralleling the rise in RNA and protein synthesis in interphase HeLa cells. Although the stacks of membranes were never seen attached to the nuclear envelope as in oocytes, they were occasionally present in the vicinity of the nucleus, and were commonly continuous with the ends of RER membranes in S and G₂ cells. Direct continuity between RER membranes and annulate lamellae has been described in HeLa cells (Epstein, 1961), oocytes (Kessel, 1968), phytohaemagglutinin-stimulated lymphocytes (Procicchiani, Miggiano & Arancia, 1968), human fibrosarcoma (Leak, Caulfield, Burke & McKhann, 1967), human bronchial epithelium (Frasca, Auerbach, Parks & Stoeckenius, 1967), and in a variety of other cell types.

Annulate lamellae may represent an intermediate state in the formation of RER as suggested by Leak et al. (1967) and Procicchiani et al. (1968), and may also function as a nucleo-cytoplasmic intermediary for establishing nuclear control over remote regions of the cytoplasm (Hsu, 1967; Kessel, 1969; Merkow et al. 1968). Bal, Jubinville, Cousineau & Inoué (1968) theorized that ribosomes are synthesized from the dense material in the annuli. Attached ribosomes were occasionally seen on annulate lamellae when they were continuous with RER membranes at one or both ends. This observation adds further impetus to the contention that these structures play a role in RER and SER formation in colcemid-treated cells (see below).

Centriole changes during the cell cycle closely paralleled those described by Robbins et al. (1968) in mechanically synchronized HeLa cells. In contrast to Robbins’s observations, the pericentriolar structures remain fairly prominent throughout early G₁, where each pair of centrioles ended up in a daughter cell. During G₁, the 2 orthogonal centrioles separate. The separated centrioles appear to replicate by budding (de Harven & Bernhard, 1956) during the S phase concomitant with DNA synthesis, resulting in 2 pairs of centrioles. Pericentriole satellites (Bernhard & de Harven, 1966) were also prominent in the S phase. These structures, as previously stressed by de Harven (1968), were only seen in the S and G₂ phases of the cell cycle and never during mitosis, and therefore could not be the site of spindle tubule attachment during mitosis. Two mature sets of centrioles are present in the cell by late G₂ in readiness for the next mitosis.

The chemistry of the centriole and related structures, and the nature of the coupling of centriole duplication to DNA synthesis, awaits further investigation. It is evident, however, that centriole replication and pericentriolar activity are in synchrony with the 4 phases of the cell cycle, and increased pericentriolar activity during late G₂ and most
of mitosis certainly suggests that centrioles play a major role in spindle tubule orientation.

Cyclic variations of HeLa cell mitochondria are very difficult to study because these organelles vary in size and shape and number in different cells, and even within the same cell. The matrix density varied, and cristae occurred in both linear and circular profiles. Mitochondrial ribosomes (André & Marinozzi, 1965), most of which were smaller (15 nm) than those in the cytoplasmic matrix, were observed in many mitochondria—especially those with a dense matrix.

Recent findings have indicated that approximately 0.2% of the DNA of mammalian cells is contained within the mitochondria (Nass, 1966). HeLa cell mitochondrial DNA (mDNA) occurs in the form of closed circular duplex molecules with a uniform length of about 5 μm. Circular dimers (10 μm) accounted for about 10% of the molecules; catenated rings of DNA molecules also exist (Hudson & Vinograd, 1967). Mitochondrial DNA occurs in the form of 3-nm fine fibrils, separate or clumped together, within cristae-free electron-lucent zones of the matrix (Nass, Nass & Afzelius, 1965). Mitochondria containing such zones were present throughout the whole cell cycle, but were slightly more prominent in $G_2$ and prophase.

Our tracer studies showed some grains over mitochondria during $S$ and rarely in $G_2$, indicating possible $^3$H-TdR incorporation. Because of the very low mDNA content and background contamination, it was not possible to determine whether or not mDNA synthesis was synchronous with nuclear DNA synthesis. In summary, we can say that it was not possible to ascertain when replication of both the organelles and their DNA takes place in the cell cycle.

Typical 20-nm ribosomes were present in all 4 phases of the cell cycle. Polysomes occurred in 3 distinct forms in the cytoplasm of interphase HeLa cells. The most common form was a tetrad, composed of 4 ribosomes. Another type consisted of 5 or 6 ribosomes arranged in circular form. Lastly, ribosomes arranged in a linear fashion, called helical polyribosomes, were occasionally seen in $S$ and $G_2$ (rarely $G_1$) cells. Polysomes in the various configurations described above, especially helical forms, have been found in a number of plant (Bartels & Weier, 1967; Wooding, 1968) and animal (Behnke, 1963; Palade, 1958; Scharff & Robbins, 1966; Waddington & Perry, 1963; Weiss & Grover, 1968) cells. Ribosomes and polysomes in all the above configurations were found in mouse plasma cell tumour isolates examined in the electron microscope after negative (phosphotungstic acid) or positive (uranyl acetate) staining (Shelton & Kuff, 1966). The helical form is characteristic of cells actively synthesizing protein. It is reasonable to assume that the various polysome configurations reflect the synthesis of different proteins during different phases of the cell cycle.

In late prophase, when protein synthesis is markedly reduced, single ribosomes were most prevalent in the cell. This situation persisted throughout metaphase and most of anaphase. In late anaphase and telophase polysomes gradually began to reform, and large numbers of polysomes were present in the early $G_1$ cell. These observations confirm those of Scharff & Robbins (1966), and Hodge, Robbins & Scharff (1969) for HeLa cells, and those of Steward & Shaeffer (1967) for Chinese hamster cells.
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Colcemid (N-deacetyl-N-methylcolchicine) (Schär, Loustalot & Gross, 1954), a synthetic analogue of colchicine was chosen to block the progress of $G_2$ cells into the next generation $G_1$ phase. Colcemid was used because it is approximately 30% less toxic than the well-known spindle poison, colchicine. Colcemid did not appear to reduce $^3$H-TdR uptake by $S$ cells, but in 2 experiments (14 and 18) the drug may have impeded progress around the cell cycle since the $G_2$ mitotic index was very low at 18 h (Fig. 1). The biochemical basis for antimitotic action is still not clearly defined (Dustin, 1963; Goldstein, Aronow & Kalman, 1968). The Golgi complexes of the interphase cells exposed to colcemid were hypoplastic and multiple. Robbins & Gonatas (1964b) also noted that the HeLa cell Golgi elements fragment under the influence of spindle inhibitors.

Colcemid-treated $G_2$ and late $S$ cells displayed a prominent increase in RER, annulate lamellae (which were usually connected to the ends of RER or SER membranes), and especially SER. Whorls and long parallel rows of multilayered SER membranes were present in most of the treated cells, but they were not present in untreated cells. Krishan, Hsu & Hutchins (1968) reported hypertrophy of RER and annulate lamellae in Earl's L cells exposed to $0.01 \mu g/ml$ of vinblastine sulphate for 6-44 h. Continuity between the membranes of annulate lamellae and the membranes of RER was also noted. The above findings, as well as ours, suggest that colcemid may stimulate interphase mammalian cells into increased synthetic activity. Wirkus & Meenakshi (1968) recently produced multilayered SER structures in meristematic cells of the root tip of Allium cepa with colchicine. George, Journey & Goldstein (1965) observed multilamellar SER profiles in HeLa cells treated with vincristine ($0.1-1 \mu g/ml$ for 1-20 h). Colchicine ($0.1 \mu g/ml$ for 12 h) induced the formation of bundles of fine filaments and some SER proliferation at a higher concentration (1 $\mu g/ml$). These changes were produced only in mitotic cells, and mainly by long exposure to a relatively high dose of colchicine.

The increased production of SER membranes in colcemid-treated HeLa cells is comparable to the hypertrophy of SER membranes produced in hepatocytes by phenobarbital (Jones & Fawcett, 1966; Remmer & Merker, 1963) and other drugs (reviewed by Stenger, 1970). Carbon tetrachloride induced similar changes in rat proximal tubule cells (Striker, Smuckler, Kohnen & Nagle, 1968). Isolated liver microsomes from phenobarbital-treated rats exhibited increased levels of drug-metabolizing (detoxifying) enzymes, as well as increased production of membrane phospholipids (Orrenius, Ericsson & Ernster, 1965). In most instances these drugs act as inducers of microsomal enzymes, but in other cases of SER proliferation there is an inhibition of microsomal metabolic activity (Stenger, 1970). The changes produced by colcemid in HeLa cells may be the morphological expression of the production of enzymes capable of detoxifying the drug, since the mitotic inhibition was reversible. Biochemical analysis of the enzymic activity of isolated HeLa cell SER membranes after colcemid treatment would be necessary to answer this question.

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Note. Autoradiography specimens are labelled AR.

Fig. 2. Toluidine blue-stained 1-μm section through a pellet of cells obtained at the time of synchronization. Metaphase, telophase, and early G₁ cells (with characteristic elongated nuclei) are evident. Note the mid-body (arrows) in the intercellular bridge. × 1040.

Fig. 3. A diplosome located in the nuclear hof of a cell in early prophase. Large numbers of microtubules are seen in the vicinity of the centriole. Patches of condensed chromatin are also evident. × 26,400.

Fig. 4. Metaphase. Chromosomes are aligned at the equatorial plate. One centriole pair (c) was intersected by the plane of the section. Most of the organelles are excluded from the spindle. Clusters of lysosomes (l), as well as fragments of nuclear envelope (arrow), are evident at the periphery of the spindle. × 5700.

Fig. 5. An oblique section through a metaphase centriole. Multidirectional arrays of spindle tubules are seen in longitudinal and cross-section. × 69,000.
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Fig. 6. Remnants of Golgi vesicles and lamellae (g) in a metaphase cell. × 16,800.

Fig. 7. Late anaphase cell. Note that some of the chromosomes have fused and are partially surrounded by nuclear envelope, especially on the sides facing the poles (arrow). Stem-bodies (sb) are evident along the cell equator. × 7,500. Inset: high-magnification micrograph of 2 stem-bodies. The increase in density (massing of small granules) of the structures occurs characteristically where the tubules from each side terminate. × 41,600.
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Fig. 8. Telophase. Note the advancing cleavage furrow. Mitochondria and stem-bodies are still present within the intercellular bridge. A small nucleolus (nu) is evident in one of the now completely formed daughter-cell nuclei. × 11500.

Fig. 9. Telophase: intercellular bridge. A mid-body occupies the central portion of the bridge which still contains a number of mitochondria. A cytoplasmic bleb (cb) is evident. (ld, lipid droplets.) × 19000.

Fig. 10. Late telophase. The daughter cells are only connected by a short bridge which is almost entirely occupied by a mid-body (mb) (g, Golgi apparatus; l, lysosomes.) × 10800.
Fig. 11. Intercellular bridge region of a telophase cell. Note the spindle tubules radiating from the dense mid-body. A centriole (c), still surrounded by a dense material, is evident in the embryonic Golgi zone of one cell. Note the polysomes at the upper left of the micrograph. \( \times 23000 \).

Figs. 12, 13. Late telophase cell mid-bodies. One end of the bridge is tenuously attached to the daughter cell. At this stage, spindle tubules are rarely seen in one of the daughter cells. \( \times 50000 \) and \( \times 46000 \), respectively.

Fig. 14. Early \( G_1 \) cell. The mid-body is no longer attached to what may be the other daughter cell (right). \( \times 34500 \).
Ultrastructure of synchronized HeLa cells
Fig. 15. Disarrayed mid-body remnants and spindle tubules at the periphery of an early G₁ cell. × 42,000.

Fig. 16. Detail of the nuclear hof region of an early G₁ cell. Both nucleoli are attached to the nuclear envelope. Few spindle tubules are present in the vicinity of the separate centrioles. Note the polysomes adjacent to the nuclear envelope. × 29,400.

Fig. 17. Early G₁ cell. A prominent Golgi apparatus surrounded by mitochondria and lysosomes occupies the periphery of the nuclear hof. Clear zones (arrow) are obvious in a number of the mitochondria. Polysomes are seen in the organelle-free regions of the cytoplasm. × 13,300.
Ultrastructure of synchronized HeLa cells
Fig. 18. Two S-cell centrioles. Procentrioles are seen budding at right angles from these structures. Note the labelled nucleus. Expt. 14 (AR). × 30 400. Inset: an S-cell centriole, surrounded by satellites (s). Expt. 14 (AR). × 64 000.

Fig. 19. Satellite (s) attached to an S-cell centriole. A microtubule can also be seen impinging upon the satellite. Expt. 12. × 63 000.

Fig. 20. Two pairs of centrioles located within the Golgi region of a late G2 cell. The centriole seen in cross-section shows the typical 9 interconnected triplet pattern. Centriole satellites are also evident. Expt. 18. × 50 000.
Ultrastructure of synchronized HeLa cells
Fig. 21. An area of nucleus and cytoplasm from a $G_2$ cell. Patches of heterochromatin and perichromatin granules (arrow) are seen in the nucleus. Fine fibrils (asterisk) are evident in the clear zones of the mitochondria. Also note the segments of RER, and the various polysome configurations. Expt. 12 (AR). $\times 20,000$.

Fig. 22. Early $S$ cell (9 h). Six grains are located in the nucleoplasm. One grain is seen over the nucleolus (rarely observed at this time). Part of a late $G_1$ cell is evident at the left. (nb, granular nuclear body.) Expt. 18 (AR). $\times 8800$. 
Ultrastructure of synchronized HeLa cells
Fig. 23. Two labelled in situ fixed S cells. The Golgi apparati (g) are well developed. Annulate lamellae (arrow) can be seen in one of the cells. Expt. 14 (AR). × 4800.

Fig. 24. Toluidine blue-stained 1-μm section through a cell pellet obtained 17.5 h after synchronization. Late S cells (LS) are clustered at the centre of the micrograph. The remaining (unlabelled) cells are either mitotic (M), G2, or second generation G1 cells. Mid S cells (S). Expt. 12 (AR). × 1090.

Fig. 25. Late S cell (17.5 h). Most of the grains are located in the vicinity of the nuclear envelope; some are also located over the nucleolus. Two fibrillar nuclear bodies can be seen in the right lobe of the nucleus. Polysomes are prominent in the cytoplasm. Expt. 12 (AR). × 7600. Inset: late S cells in a toluidine blue-stained section from the same pellet. The grains are distinctly located over the nuclear envelope and the nucleolus. × 1500.
Fig. 26. A small portion of a typical $G_1$ cell nucleus. The nucleolus is composed of both a granular and a dense fibrillar (f) component. A fibrillar nuclear body (arrow) can be seen above the nucleolus. Expt. 14 (AR). $\times 24,000$. Inset: high-magnification micrograph of the nuclear body, which appears to be composed of intertwined densely packed fibrils. $\times 93,000$.

Fig. 27. Late $G_1$ cell (9 h). Note the 3 fibrillar nuclear bodies. Expt. 18 (AR). $\times 24,000$.

Fig. 28. A higher-magnification micrograph showing both a granular and a fibrillar nuclear body. None of the grains are associated with these structures. Expt. 12 (AR). $\times 44,000$. 

Fig. 29. G2 cell mitochondrion. Mitochondrial ribosomes (large arrow) are seen in the dense matrix. Various polysome configurations, some of which are circled, including a helical polysome (small arrow), are also evident. (AR). ×62,500. Inset: typical helical polysome. ×93,000.

Fig. 30. This micrograph illustrates the end-to-end attachment of annulate lamellae to RER in the cytoplasm of an S cell. ×76,000.
Ultrastructure of synchronized HeLa cells
Fig. 31. Photomicrograph of a culture fixed and stained (Feulgen method) after a 4-h exposure to colcemid. Prominent C-metaphases are seen (asterisks). ×1140.

Fig. 32. Colcemid-treated G2 cell (18 h). The nucleolus is attached to the nuclear envelope. A fibrillar nuclear body can be seen in the nucleus (arrow). Note the abundant polysomes and the arrays of SER (asterisk). Expt. 14 (AR). ×10400.

Fig. 33. Colcemid-treated G0 cell. Note that the Golgi apparatus (g) are small and multiple. Expt. 17 (AR). ×10800.

Fig. 34. Centriole (colcemid-treated G0 cell). The fine structure of the centriole and related structures are unaffected by the drug. Occasional microtubules can still be seen in the cytoplasm. Expt. 18 (AR). ×48000.
Fig. 35. Typical cytoplasmic changes produced by colcemid. Note the continuity between the SER and annulate lamellae (al), SER and RER, and RER and annulate lamellae. Oriented SER lamellae (ser) are also seen. (g, Golgi; l, lysosomes.) × 20000. Inset: detail of a portion of the annulate lamellae in the same cell. Small dense granules (and fibrils) cluster around the annuli. × 60000.

Fig. 36. A whorl of SER in a colcemid-treated cell. Note the glycogen particles (large arrow) and the helical polysome (small arrow). Expt. 17. × 36000.
Ultrastructure of synchronized HeLa cells