EFFECT OF COLCEMID ON THE CENTRIOLE CYCLE IN CHINESE HAMSTER OVARY CELLS

RYOKO KURIYAMA
National Institute for Basic Biology, Okazaki 444, Japan

SUMMARY

The structural changes in the centrioles in Chinese hamster ovary cells were monitored by electron microscopy of whole mount preparations to investigate the effects of colcemid on the events in the centriole cycle. The population of mitotic cells increased with time of incubation with colcemid, but the arrest at mitosis by this drug was soon overcome, resulting in the formation of nuclei and a change in the shape of the cells again spreading over the substrate. The maximal mitotic index was reached every 25 h in the presence of either 0.10 or 0.91 µg/ml of colcemid. During this time, cells became multinucleated, increased greatly in size, and accumulated 8 to 10-nm filamentous bundles in the cytoplasm instead of microtubules, almost all of which had been depolymerized after exposure to colcemid.

In the cells that were continuously treated with colcemid, a pair of centrioles became disoriented and each subsequently produced a daughter centriole. However, these daughter centrioles elongated to only half their full length; many unusual figures in the centriolar pairs resulted from their proceeding normally to the phases for disorientation and nucleation for centrioles in the next cycle. Although the rate of centriole elongation and the frequency of formation of the daughter centrioles were decreased by increasing the concentration of colcemid, the disorientation of the centrioles was not disturbed by this drug. The inhibitory effect of colcemid on centriolar nucleation and elongation was found to be totally reversible; the formation and elongation of new daughter centrioles occurred again just after removal of the drug.

Prolonged treatment of cells with colcemid caused ultrastructural changes in the centrioles, such as the outgrowth of microtubules from the wall of centriolar triplets or the formation of unusual bundles of microtubules around the centrioles.

INTRODUCTION

In animal cells, the progression of centriolar events through the cell cycle is tightly coordinated with other cellular events. The number of centrioles in each cell is under very strict control, and neither too many nor too few centrioles are observed. Almost all observations concerning centrioles have had to rely on electron microscopy of thin sections, despite its time-consuming procedure.

A recent advance in technique had allowed the visualization of centrioles in mammalian cultured cells by electron microscopy of whole mount preparations (Kuriyama & Borisy, 1981), and has made it possible to re-examine the structural changes in centrioles as a function of the cell cycle (Kuriyama & Borisy, 1981). A method was developed to document the centriole cycle in a graphic form, in which centriolar profiles were placed in six categories according to their orientation and the ratio of length of daughter centrioles to that of parents. The morphological changes in the centriole cycle were characterized by the three distinct events of nucleation, elongation
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and disorientation. The proportion of centrioles in each category was then plotted as a frequency histogram in which the centriolar events were represented. The results obtained from the whole mount preparations of lysed Chinese hamster ovary (CHO) cells fully confirmed the pattern of events in the centriole cycle as determined by electron microscopy of thin sections (Stubblefield, 1968; Robbins, Jentzsch & Micali, 1968; Rattner & Phillips, 1973). This is summarized in Fig. 1. Further application of this whole mount method had already demonstrated the effects on the cycle of several treatments, such as inhibition of DNA synthesis or enucleation (Kuriyama & Borisy, 1981).

Fig. 1. Diagram of the 6 categories of centrioles, which are arranged according to their orientation and the length ratio of daughter to parent centrioles. Category I represents profiles of 2 centrioles with full-sized parent and full-sized or almost full-sized daughters, but not in orthogonal configuration. In categories II through VI, the parent and daughter centrioles are situated perpendicular to each other and the ratio of length of daughter to parent centrioles increases: II, 0-0-0-2; III, 0-2-0-4; IV, 0-4-0-6; V, 0-6-0-8; VI, 0-8-1-0. The progress in the centriolar profile from I to II represents the nucleation of the daughter centrioles; categories II through VI represent the elongation of the daughter centriole; and from VI to I represents disorientation.

The lower diagram shows the centriole cycle, in which the morphological changes of centrioles are characterized by 4 distinct events. Daughter cells formed by cell division receive a pair of orthogonally arranged, almost full-sized centrioles which become disoriented in G1-phase. During late G1 or early S, a short daughter centriole appears at the proximal end of each parent, oriented perpendicularly. The daughter centrioles elongate slowly from S to G2-phase and attain almost full size at prophase, when the 2 pairs of centrioles separate and begin to migrate towards opposite ends of the nucleus. They are positioned at each spindle pole and are segregated to each daughter cell by mitosis (M), after which a new centriole cycle is begun.
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Colchicine and its derivative, colcemid, are well known inhibitors of mitosis that interfere with the structure of the mitotic spindle; they have long been used for obtaining synchronized mitotic cell populations (Stubblefield & Klevč, 1965). The molecular mechanism of the inhibition is based upon their specific binding to the microtubule protein, tubulin (Taylor, 1965; Borisy & Taylor, 1967). Therefore, these alkaloids cause disruption of the cytoplasmic microtubules as well as prevention of tubulin assembly into microtubules. Recent work done in vivo showed us the detailed mechanism of colchicine inhibition on microtubule assembly. Margolis & Wilson (1977) suggested that colchicine binds first to soluble tubulin dimer, which then adds to the growing microtubule as a colchicine–dimer (CD) complex and effectively ‘caps’ the microtubule and aborts further polymerization. Insensitivity of the centriole to these drugs is well documented, but few reports have been presented on the structural events in the centrioles after exposure to colchicine or colcemid. The research described in this paper is concerned with the effects of colcemid on the centriole cycle and its coordination with nuclear events in CHO cells.

MATERIALS AND METHODS

Cell culture and synchronization

All experiments were performed on Chinese hamster ovary cells grown as monolayer cultures, as described in a previous paper (Kuriyama & Borisy, 1981), with some modifications. CHO cells were maintained in Ham's F-12 medium (Nissui Seiyaku Co., Ltd, Tokyo, Japan) supplemented with 10% foetal bovine serum (Flow Laboratories, Stanmore, N.S.W., Australia), antibiotics and 15 mM-N,N-diethyl-N-methyl-2-hydroxyethylpiperazine-N’-2-ethane sulphonic acid (HEPES) at pH 7.2 in a humid atmosphere with 5% CO₂ at 37 °C. The cells had a mean generation time of 17 h as determined by periodic cell counts in several particular areas.

Synchronization of cells at S- and M-phase was performed as described in the previous paper (Kuriyama & Borisy, 1981). Cells at G1 stage were obtained by the addition to a non-confluent culture of thymidine to a final concentration of 2-10 mM. After 10–15 h, the monolayers were then washed free of thymidine and returned to fresh medium. In order to get mitotic cells, the cells were kept in culture in fresh medium for 4–5 h after removal of thymidine. Then colcemid was added at a concentration of 0-10 μg/ml and the cells cultured for an additional 6–7 h until the mitotic index was at a maximum value (40%; refer to curve A in Fig. 2). Cells arrested at mitosis by colcemid were collected by centrifugation at 500 g for 3–5 min, and then plated into new dishes for further culturing with colcemid for each experiment.

Preparation of cells for determining and mitotic index and nuclear number in colcemid-treated CHO cells

After culture of synchronized cells for the desired period, they were collected by direct scraping off from the plastic dishes with a rubber policeman. The cells were then pelleted in a centrifuge and resuspended in a medium containing 10 mM-PIPES (1,4-piperazine-N-N’-bis-(2-ethane sulphonic acid)), 0.5 mM-MgCl₂, and 1 mM-EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetate) at pH 6.7. Under these hypotonic conditions, the cells swelled and did not lyse, favouring clearer observations of chromosomes or nuclei within the cells by phase-contrast microscopy.

Preparation and visualization of centrosomes from colcemid-treated cells

Interphase or mitotic cells were lysed as described previously (Kuriyama & Borisy, 1981). Sedimented cells were resuspended for 1–2 min in 10 vol. distilled water, then lysed in a
solution of 2% Triton X-100 and 1 mM-PIPES by the addition of an equal volume of 4% Triton X-100 in 2 mM-PIPES at pH 6.7. As reported (Kuriyama & Borisy, 1981), the nucleus becomes increasingly swollen and pale over the time of incubation at room temperature, as observed under phase-contrast microscopy; this process makes it possible to visualize centrioles attached to the nucleus, by electron microscopy of whole mount preparations. After 20–50 min, when the nuclei were judged to be sufficiently pale, 0.5 vol. of 3% glutaraldehyde in distilled water was added to stop the extraction of the nucleus. Colcemid-treated cells showed a small increase in the number of nuclei that retained no centrosomes. Free centriolar profiles were counted for making distribution histograms. In the case of mitotic cells, free centrosomes were produced after lysis of cells because of the absence of nuclei; therefore, glutaraldehyde fixation was done immediately afterwards.

**Microscopy**

Whole mount samples were prepared as described by Gould & Borisy (1977) with some modifications (Kuriyama & Borisy, 1981). Several drops of fixed samples were sedimented onto ionized Formvar-coated 400-mesh grids, which had been heavily coated with carbon. After washing with distilled water, grids were stained with 2% phosphotungstic acid with its pH adjusted to 6.5 with NaOH, and examined in a JEM 100 CX or Hitachi H-500H electron microscope operated at 75–100 kV.

Frequency histogram profiles of centriolar configurations were constructed by photographing and measuring the arrangement and the length of the daughter and parent centrioles. They were then classified into one of 6 categories (Fig. 1) as described in detail in the previous paper (Kuriyama & Borisy, 1981). In order to construct a frequency histogram for one sample, 100–150 photographs of centrioles or centrosomes were taken. The set of histograms in each figure represents experiments run in parallel in the same culture.

For preparation of thin sections, samples were fixed with 2.5% glutaraldehyde, postfixed in 1% OsO4, and stained with 0.5% uranyl acetate for 2 h at room temperature. After dehydration through an ethanol series, they were infiltrated and embedded in the Epon formulation of Spurr according to the standard procedure. Thin sections were cut on a Sorvall Porte-Blum II Ultramicrotome with glass knives, picked up on Formvar-coated 200- or 400-mesh grids and stained with uranyl acetate and lead citrate.

**RESULTS**

**Effect of prolonged culture of CHO cells with colcemid**

In order to obtain cells synchronously arrested at mitosis, 0.10 µg/ml of colcemid was added to the cell culture as described in Materials and Methods. The population of the mitotic cells increased with time of incubation (Fig. 2, curve A), but this blockage at mitosis was found not to be permanent. When the mitotic index reached a maximal value (40%) at 10 h after removal of thymidine, floating cells in the medium were collected and placed into new dishes to continue further culturing of cells with colcemid. Cells eventually became nucleated and spread over the substrate. In Fig. 2, curves B and C, the number of cells with condensed chromosomes was plotted against the time of incubation with 0.10 and 0.91 µg/ml of colcemid, respectively. At 25 h, nuclei could be detected in over 90% cells in the populations; thereafter they advanced to the mitotic phase, with the second maximal mitotic index appearing about 13 h later. No difference in the effect of colcemid concentration between doses of 0.10 and 0.91 µg/ml was observed until around 50 h. The third mitotic cycle actually appeared less clearly in the presence of 0.91 µg/ml of colcemid. Few cytoplasmic microtubules in these cells were detected by electron microscopy, so it is suggested that microtubules
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may not participate in the attachment and spreading of colcemid-treated cells over the substrate.

Fig. 3 presents the change in nuclear number within one cell upon incubating the cells in a medium containing 0.10 μg/ml of colcemid. It is evident from these histograms that a single nucleus was found in over 70% of cells after the first mitotic stage in colcemid solution. Further culturing resulted in the formation of many multinucleated progeny, at which time not only cellular size but also the form of spreading over the substrate were more or less changed. In the control, attached and spreading cells on the plastic dishes had a rather long and slender asymmetric shape (Fig. 4A), while colcemid-treated cells seemed to lose their stretched appearance and to flatten into a relatively uniform sheet (Fig. 4B). As shown in Fig. 4C, blocked cells also became larger as the nuclear number increased.

![Fig. 2. Change in the mitotic index according to the time of incubation with colcemid in CHO cell cultures. Curve A, at 4 h after removal of thymidine (arrow), 0.10 μg/ml of colcemid was added to block the cells at mitosis. When a maximum mitotic index about 40% was obtained at 10 h, mitotic cells were collected and placed into new culture dishes which contained 0.10 μg/ml (curve B) or 0.91 μg/ml (curve C) of colcemid.](image)

Changes in centriolar profile of CHO cells in the presence of colcemid

The centriole cycle in colcemid-blocked cells. As described in a previous paper (Kuriyama & Borisy, 1981), centrosomes in mammalian cultured cells attach firmly to the nucleus. The profile of the centriolar pair on the nucleus can be easily visualized by extracting the isolated nucleus–centrosome complex with Triton X-100 in a medium of low ionic strength. This method was also applicable to the cells being kept in culture with colcemid for a long time, since this complex was found to be quite stable even in the presence of colcemid, in spite of the tendency for increased numbers of free centrosomes in blocked cells.

Frequency histograms show many changes in the profiles of centrioles as the time of incubation with colcemid increased. This is summarized in Fig. 5, which represents...
Fig. 3. Change in the nuclear number in CHO cells during culture with colcemid. After treatment with thymidine for 12 h, cells were washed and transferred to the fresh medium at zero time. Colcemid (0.10 μg/ml) was added at 5 h, and the culture of the collected mitotic cells was continued in the same medium. Incubation times in hours are shown on the right.

the centriole cycle of CHO cells starting from M-phase, through incubation with 0.10 μg/ml of colcemid, until they were lysed. Fig. 5A shows the centriole profile of rounded cells in mitosis obtained 7.5 h after washing out of thymidine, and 2.5 h after addition of colcemid. The most abundant categories were VI and I, that is two almost full-sized centrioles either in an orthogonal configuration relative to each other (VI), or not (I). When the mitotic cells gathered at 10 h after removal of thymidine
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were plated and maintained in fresh medium with 0.10 μg/ml of colcemid, the profile distribution of centrioles changes as shown from B through I. After 13.4 h of culture (Fig. 5 B), category VI declined dramatically, and over 60% of the profiles shifted to category I. This indicates that disorientation can occur normally even in the presence of colcemid. At about 25 h, when almost all of the cells had regained nuclei (refer to Fig. 2), the centriolar profile of categories II and III as well as I became predominant (Fig. 5 C). Since the centriole could pass into category I from II, it is concluded that nucleation of the daughter centriole is not inhibited by this drug.

Further culturing of the cells with colcemid caused the peak in the frequency histogram to shift to higher-numbered categories, indicating the elongation of centrioles (Fig. 5 D). In fact, nearly 60% of the profiles of centrioles harvested from the colcemid-treated cells were in category III at 29.7 h. Though the elongation of daughter centrioles continued in cultures maintained in the medium containing 0.10 μg/ml of colcemid, the rate of this progression was apparently slowed down, as shown in Fig. 5 E to I, where the distribution histograms indicate no further advancement. Even up to 56.7 h in culture, the most abundant classes of centriolar profiles were still categories III and IV, corresponding to a daughter/parent ratio of 0.2–0.6. However, other events in the centriole cycle such as disorientation and nucleation were able to proceed normally.

The observation of centrioles in prolonged culture of CHO cells with 0.10 μg/ml of 0.91 μg/ml of colcemid provided many chances of encountering figures like those shown in Fig. 6 A and B. These negatively stained electron micrographs of centriolar pairs, prepared from colcemid-blocked cells at 15–18 h, show the uneven length of parent centrioles in each pair; this has never been observed in control cells. This configuration apparently resulted from the orthogonal formation of grand-daughter, or third generation centrioles from each parent and daughter; the daughter is already

Fig. 4. Phase-contrast light micrographs of an exponentially growing asynchronous culture of CHO cells (A); collected mitotic cells treated with 0.91 μg/ml of colcemid for 13.5 h (B); and 0.10 μg/ml for 63.0 h (C). A, ×180. B, ×180. C, ×300.
not in perpendicular configuration to the parent, but is only partially elongated because of the inhibitory effect of colcemid on centriole elongation. It might be concluded that complete elongation of the daughter centriole is not indispensable for the granddaughter's nucleation. However, it seems worth mentioning here that the ratio of lengths of daughter to parent centrioles is always slightly larger when grand-daughters

Fig. 5. Frequency histograms of centriole profiles in CHO cells treated continuously with 0.10 μg/ml of colcemid. Ordinate: frequency in percentage; abscissa: category of centriole profile. After removal of thymidine at zero time, 0.10 μg/ml of colcemid was added at 5 h to block the cells at M-phase. Isolated mitotic cells were placed into new culture dishes with 0.10 μg/ml of colcemid and kept in culture until they were lysed. Times of incubation shown on right, in hours. Times in parenthesis are after isolation of mitotic cells.
are present than the ratio of lengths in category III. At the same time, abnormal pairs of centrioles become conspicuous, as illustrated in Fig. 6c. The daughter centriole (arrow) can be observed to nucleate from the longer parent at right angles, but not to nucleate from the shorter parent. It seems possible that nucleation in the centriole cycle cannot occur from a parent centriole that is too short. Though no direct evidence to verify this idea is now available, the result suggests that a critical length of parent centriole is necessary for new centriole formation.

Fig. 6. Whole mount electron micrographs of centrioles obtained from CHO cells treated with 0.06 μg/ml of colcemid for 15 h (A) or with 0.10 μg/ml for 18 h (B) and 25 h (C). Note the short centriole (arrow) in C. A, x 40500. B, x 41900. C, x 40500.

Inhibition of daughter centriole elongation by colcemid. That the primary effect of colcemid is to inhibit elongation of daughter centrioles was also confirmed by a separate experiment, in which the investigation of the effect of colcemid on the centriole cycle was begun from synchronized S-phase cells. Results are shown in Fig. 7. After blockage of the cells with 5 mM-thymidine for 10 h (Fig. 7A), the centriolar profiles were found to be clustered in categories II, III and IV, where the centriole pairs contain daughters less than 60% of full length. When the cells were washed free of thymidine and cultured in fresh medium for 7 h, the peak in the histogram moved towards longer daughter centrioles. Also centrioles in category I began to increase as shown in Fig. 7B. This histogram should be compared to the ones shown in C and D, in which the cell were cultured in media containing 0.10 and 0.91 μg/ml of colcemid, respectively, after removal of thymidine. In spite of an advance in the elongation of daughter centrioles in colcemid-treated cells, the delay in the progress of the cycle is remarkable. The higher the concentration of colcemid, the more evident the delay in the elongation process.

Inhibition of centriole nucleation by colcemid. It was also found that higher concentration of the drug caused the frequency of daughter centriole nucleation, as well as the rate of elongation, to decrease. Fig. 8 shows the change in profile distribution of centrioles harvested from cells cultured with various concentrations of colcemid. After
exposure of collected mitotic cells to 0.06 μg/ml of colcemid for 17 h, the most abundant class of centriolar profiles was category II (Fig. 8A). With increasing concentration, however, delay in the progression of the centriole cycle became obvious (Fig. 8B to D). Finally, formation of the daughter centriole is not detectable at all in the cells treated with 0.91 μg/ml of colcemid. At the same time, elongation of the daughter centriole itself appeared to stop immediately as shown in D, where the profile distribution was quite similar to the mitotic cells employed here as starting material. This implies no more progress in the centriole cycle. In spite of these potent selective effects of colcemid on nucleation and elongation, the centriolar event of disorientation was not itself directly disrupted by this drug as far as we observed. Therefore, a centriolar pair appeared, composed of a parent and daughter each existing in a separate configuration. In fact, attempts were unsuccessful to detect any regularly perpendicular arrangement between the centrioles after 23 h culture of cells with 0.91 μg/ml of colcemid.

Fig. 7. Change in the frequency histograms of centriole profiles in CHO cells upon starting colcemid treatment from S-phase. After treatment with 5 mM-thymidine for 10 h, cells were washed and cultured for 7 h in fresh medium, which contained: A, 0 μg/ml; B, 0.06 μg/ml; C, 0.10 μg/ml; D, 0.91 μg/ml of colcemid. A. The centriole profiles of cells just after removal of thymidine.

Fig. 8. Change in the frequency histograms of centriole profiles in CHO cells in the presence of various concentrations of colcemid. After isolation, the mitotic cells continued in culture for 17 h with: A, 0.06 μg/ml; B, 0.10 μg/ml; C, 0.13 μg/ml; D, 0.91 μg/ml of colcemid.
Due to the inhibitory effect of colcemid on nucleation and elongation, mitotic cells obtained after prolonged treatment with colcemid came to have different centriolar profiles than normal mitotic cells. However, when centrosomes containing the centrioles with abnormal profiles were tested in an *in vitro* preparation of brain microtubules by the method developed by Gould & Borisy (1977), their nucleating activity was as high as those in normal mitotic cells.

Reversibility of the inhibitory effect of colcemid on centriole nucleation and elongation. Fig. 9 shows the reversible effect of colcemid on the centriolar events of nucleation and elongation. Synchronized *M*-phase cells were placed into culture dishes with 0.91 μg/ml of colcemid. Fifteen hours later the drug was washed out and the cells were returned to fresh medium. Fig. 9A illustrates the frequency distribution of centrioles in the cells just prior to removal of colcemid. As before, the profiles were clustered in categories V, VI and I, and also most of the pairs were disoriented regardless of the length of daughter centrioles. After 2.5 h culture in fresh medium (Fig. 9B), the peak in the histogram shifted to category II, indicating the occurrence of centriole nucleation. As shown in Fig. 9C, further culturing of the cells permits these daughter centrioles to elongate. This distribution should be compared to the one shown in D, which presents the profiles of centrioles obtained from the cells cultured in a colcemid-containing medium for the same duration as those in C. This histogram was essentially the same as the control illustrated in A. Therefore, it may be concluded that the
inhibitory effect of colcemid on the centriole cycle is completely reversible, at least in terms of centriolar nucleation and elongation.

**Ultrastructural changes in CHO cells by colcemid treatment**

As described above, longer-term incubation of cells with colcemid caused several structural changes in CHO cells. The fine ultrastructural change in cells at 27.5 h after addition of 0.1 mg/ml of colcemid was easily characterized from sectioned electron micrographs. In treated cells almost all cytoplasmic microtubules depolymerized. Instead, a massive accumulation of filamentous bundles became prominent. They formed discrete large bundles of aligned 8 to 10-nm filaments in the cytoplasm of each cell (Fig. 10A, B). Although this filamentous structure appeared most conspicuously in the cytoplasm of interphase cells, abundant filaments running through the cytoplasm could also be observed within the cells at the mitotic stage (Fig. 10D).

![Fig. 10. Thin-section electron micrographs of colcemid-treated CHO cells with nucleus (A, B, C) or chromosomes (D). Arrows in A show the bundle structure of filaments. B shows a high magnification of the area outlined in A. A filamentous bundle near the centriole is illustrated in C. A, × 3400. B, × 20,000. C, × 51,800. D, × 10,800.](image)

Distinct changes in centriolar ultrastructure were also produced by colcemid. Some representative electron micrographs of whole mount preparations of treated cells are presented in Fig. 11. Fig. 11A shows the outgrowth of a microtubule from the wall of centriolar triplet microtubules. In some cases, a triplet (b) or doublet microtubule (c) and plural microtubules from different triplets (c) were detected jutting out from different triplets. The outgrowing microtubules are stable, unlike cytoplasmic ones, since conditions employed here in whole mount preparations to visualize centrioles
attached to the nuclei made cytoplasmic microtubules depolymerize completely. Stable microtubules were also observed near centrioles as bunching clusters (Fig. 11 D, E, F). We do not have any data to relate these short bundle of microtubules to the abnormal nucleating form of daughter centrioles formed under the influence of colcemid. However, we did have many chances to detect several kinds of abnormal combinations of centrioles (Fig. 11 G to I).
DISCUSSION

The present results suggest that, even in the presence of colcemid, CHO cells retain their ability to go through their own specific nuclear cycle after overcoming the arrested metaphase caused by this alkaloid. The same endogenous recovery from colchicine or colcemid has already been demonstrated in a variety of organisms (Stubblefield, 1964; Kleinfeld & Sisken, 1966; Rosenbaum & Carlson, 1969; Fragata, 1972; Wunderlich & Heumann, 1974). In their report on cilia regeneration in Tetrahymena, Rosenbaum & Carlson (1969) observed many vacuoles with yellow crystals in the cell coincident with the recovery from the colchicine-induced arrest; they finally postulated that the overcoming of the colchicine inhibition in Tetrahymena was probably a unique phenomenon related to the feeding and excretory mechanisms of this organism. Cultured mammalian cells (Stubblefield, 1964; Kleinfeld & Sisken, 1966) including the CHO cells described in this report, however, appeared to retain a considerable amount of colcemid in the cytoplasm, sufficient to suppress any formation of cytoplasmic microtubules. We do not have any data to explain reasonably the cellular control mechanism for recovery from colcemid blockage, but in vitro experiments suggest a possibility that colchicine inhibition on microtubule assembly is not absolute (Sternlicht & Ringel, 1979; Farrell & Wilson, 1980). Some free tubulin dimer addition occurs over the colchicine–tubulin dimer block to form copolymer, eventually resulting in recovery from the effect of the block. Further studies are awaited for elucidation.

Graphic representation of the centriole cycle provides us with a means for determining the specific effects of colcemid on each centriolar event. It is elongation, rather than other events in the centriole cycle, that is preferentially inhibited by colcemid. This results in the perpendicular formation of a short daughter centriole from the parent that is not full-sized. Therefore, it might be concluded that the several centriolar events can occur, to some extent, independently of each other. However, the selective effects of colcemid on the centriolar structure were found to be concentration-dependent: culturing the cells with 0.91 μg/ml of colcemid produced interference not only with elongation, but also with nucleation itself. The potent ability of centrioles to replicate and mature in the presence of colcemid (Stubblefield, 1968) may be due, at least in part, to the drug concentration used in the experiments. In spite of the aforementioned effect of colcemid on the centriole cycle, there are a few reports observing the considerable number of centrioles produced in the pituitary (Hubert, Flament-Durand & Dustin, 1974; Flament-Durand, Hubert & Dustin, 1976) by the incubation of dissected tissues with microtubular poisons such as colchicine or vincristine: this treatment also led to ciliogenesis or formation of macrotubules and crystalline structures within the cells. Since centriolar formation appears to be greatly influenced by the conditions used in each experiment, involving such variables as dehydration (Hubert et al. 1974; Flament-Durand et al. 1976), Tris-buffer medium (Margulis, Banerjee & White, 1969) or drug concentration, it is conceivable that these apparently contradictory phenomena in pituitary and tissue culture cells resulted from different sensitivities to treatment in each specific cell.
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Much has been observed concerning the morphological changes in cells after exposure to microtubular poisons. At the cellular level, these drugs cause disruption of cytoplasmic or spindle microtubules, resulting in an apparent increase in the number of 10-nm filaments similar to that reported in colchicine-treated cells (Ishikawa, Bischoff & Holtzer, 1968; Wisniewski, Shelanski & Terry, 1968; Krishan & Hsu, 1969; Olmsted et al. 1970; Goldman, 1971). A recent paper by Pytela & Wiche (1980) suggested that high molecular weight polypeptides (HMWPs) prepared from the fraction of intermediate filaments might have a dual role in the cell, serving not only as regulators of microtubule assembly but also as linker components between microtubules and intermediate filaments. Therefore, it seems highly probable that after destruction of microtubules by colcemid, the remaining HMWPs caused the 10-nm filaments to associate with one another, consequently forming discrete large filamentous bundles in the cytoplasm. The most prominent alteration in the centriole revealed by electron microscopy of whole mount preparations was the growing out of microtubules from the triplet microtubules on one side of the centriole. It is not clear whether this outgrowth results from colcemid disturbing the programming for centriole construction; however, the same morphological change has already been reported (Zuckerberg & Solari, 1973) in the seminiferous epithelium of the mouse after treatment with vinblastine.

It is well known that, irrespective of their morphological similarities, microtubules obtained from various sources show a remarkable difference in their structural stability (Behnke & Forer, 1967). Generally, cytoplasmic microtubules are very labile, being easily disassembled by treatments such as low temperature or high pressure. On the other hand, microtubules from motile structures or from the centriole are relatively stable and resistant to those treatments. Colchicine or colcemid prevents tubulin assembly into cytoplasmic microtubules both in vivo and in vitro, but these drugs are never able to depolymerize ciliary, flagellar or centriolar microtubules.

As for the effect of these drugs on the assembly of stable microtubules, however, several investigators have already reported that ciliary and flagellar regeneration could not proceed in the presence of the alkaloids in Chinese hamster fibroblasts (Stubblefield & Brinkley, 1966), Chlamydomonas reinhardii (Rosenbaum, Moulder & Ringo, 1969), Tetrahymena pyriformis (Rosenbaum & Carlson, 1969), Stentor coerulesus (Neviackas & Margulis, 1969) or in sea urchin embryos (Iwaikawa, 1977). They postulated that it was the potent binding activity of the drugs to tubulin, the microtubule subunit protein (Taylor, 1965; Borisy & Taylor, 1967), that interfered with regeneration of cilia or flagella by preventing the assembly of tubulin into motile microtubules. Results obtained in this work also demonstrate an inhibitory effect of colcemid on centriole formation in CHO cells. Therefore, it seems plausible that the stable ciliary and flagellar as well as centriolar microtubules are generated from colcemid-sensitive tubulin subunits sharing an identical unit for labile cytoplasmic microtubules. Furthermore, we have already obtained some evidence concerning the instability of reconstituted microtubules from ciliary and flagellar outer-doublet tubulin (Kuriyama, 1976). Differences in structural stability might not be due to the characteristics of the tubulin molecule itself. Rather, they could be due to the properties of the additional supporting substances surrounding the motile or centriole microtubules, or some
conformational changes occurring in tubulin subunits just at the time of formation of the cilia, flagella or centrioles in vivo.

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REFERENCES


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