
MORPHOLOGICAL AND KINETIC ASPECTS OF MITOTIC ARREST BY AND RECOVERY FROM COLCEMID

RUTH G. KLEINFELD and JESSE E. SISKEN

From the Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, New York, and the Department of Biology, City of Hope Medical Center, Duarte, California

ABSTRACT

The effect of Colcemid on the *in vivo* system of regenerating rat liver and on the *in vitro* system of HeLa cell cultures was studied to determine some of the morphological and kinetic aspects of metaphase blockage and recovery. The results indicated that under certain conditions the blocking effects of the drug were reversed; a functional bipolar spindle reorganized, and normal division resulted. Individual HeLa cells were followed by time-lapse cinemicrography prior to, during, and after Colcemid treatment. There was no apparent effect on cells in interphase. Cells entered mitosis at a normal rate and passed through prophase. A spindle was formed in most cells, albeit deformed, stunted, or shrunken. Within a certain range of drug concentrations, the spindle lengths showed characteristic unimodal distributions. After a 2-hr exposure to the drug followed by 1 hr in fresh medium, spindle lengths were restored to normal. Cells arrested in metaphase for periods as long as 5 hr were capable of reconstituting a normal functional spindle. Cells blocked for periods longer than 5 to 6 hr failed to recover.

INTRODUCTION

Colchicine and its derivatives arrest mitosis in metaphase, presumably by causing a disorientation of the submicroscopic organization of the mitotic apparatus. In the absence of an organized spindle the chromosomes often disperse and lie scattered throughout the cell, forming the classical *c*-mitosis. The cell may remain in this arrested state for several hours and then revert to interphase, with the formation of several karyomeres which subsequently fuse to produce large polyploid nuclei, or it may degenerate. The concentration of the drug,

and duration of exposure to it determine the pattern of arrest and recovery (4, 33, 12, 17, 9).

The present study deals with the action of Colcemid on the *in vivo* system of regenerating rat liver and the *in vitro* system of HeLa cells. Colcemid (Ciba) has been shown to be less toxic and more effective than colchicine as a metaphase-blocking agent for animal cells (10, 24, 25). Some of the morphological and kinetic aspects of metaphase blockage and recovery were investigated. The results indicate that under certain conditions

a large percentage of blocked metaphase cells recover to complete mitosis: that is, the inactivating effects of Colcemid upon the mitotic apparatus are reversed, a functional bipolar spindle is reorganized, and normal division occurs.

MATERIALS AND METHODS

A. Regenerating Liver

Young adult male Sprague-Dawley rats were subjected to partial hepatectomy (16). 25 hr after surgery, when the first burst of mitosis is reaching a peak, a single intraperitoneal injection of Colcemid at a dose of 0.1 mg/100 g body weight was administered. (Colcemid was kindly supplied by Dr. Philip C. Eisman, Ciba Research Department, Summit, New Jersey). Liver samples were taken 4, 6, 8, 11, 20, and 24 hr. following Colcemid injection, fixed in alcohol:acetic acid (3:1) or 10% neutral formalin, and embedded in paraffin. Sections of various thicknesses were prepared and staining procedures such as the Feulgen reaction, Heidenhain's iron hematoxylin, and azure B at pH 4.0 were used to facilitate analysis of spindle aberrations and nuclear reconstruction patterns.

B. Tissue Culture

Monolayer cultures of HeLa and human amnion cells were grown on cover slips in Leighton tubes and in Rose chambers. After a good monolayer was established and the cultures were growing exponentially, the cultures were treated with colchicine or Colcemid at concentrations varying from 1×10^{-6} to 5×10^{-5} mg/ml of growth medium. Cultures grown on cover slips were fixed in osmium tetroxide vapors for cytological examination of various stages of metaphase arrest and subsequent recovery. Rose chamber cultures were used for time-lapse cinemicrographic studies.

RESULTS

A. Regenerating Liver

In all liver samples taken 4 or 6 hr following Colcemid administration, 98 to 100% of the dividing cells were arrested in metaphase. Samples taken 8, 11, 20, and 24 hr after drug administration revealed two distinct patterns of recovery.

(1) In most animals, liver cells entering mitosis continued to be blocked in metaphase even 20 hr after Colcemid administration, i.e., essentially no anaphase and telophase figures were seen. Cells containing three or more karyomeres or bizarre-

shaped, highly polyploid nuclei were common in the 20- and 24-hr samples. This represented the typical *c*-mitosis recovery pattern (Fig. 1 A).

(2) In some liver samples taken 8, 11, or 20 hr after Colcemid administration, increased numbers of anaphase and telophase figures were found. Certain mitotic aberrations, such as tripolar spindles and groups of chromosomes lying outside the main spindle area at anaphase and telophase, were occasionally seen. Relatively few cells with karyomeres or highly polyploid nuclei were found in 20- and 24-hr samples (Fig. 1 B).

This second pattern of recovery suggested that under certain conditions the Colcemid-induced disorientation of the mitotic apparatus could be reversed so that, even following a prolonged arrest at metaphase, a functional spindle could be reconstituted and a relatively normal division take place.

The possibility existed that what we interpreted as the second pattern of recovery was in actuality that of an occasional animal more tolerant of the dose of Colcemid used, and that most cells entering mitosis were never blocked at metaphase. Samples taken 11 to 24 hr after treatment would, therefore, resemble untreated controls. This possibility was ruled out by an experimental series in which liver biopsies were taken from each of 15 animals at 4, 11, and 24 hr after Colcemid was given. Two animals of this group showed the second pattern of recovery. Examination of the 4-hr biopsy samples left no doubt that essentially all cells entering mitosis were blocked at metaphase.

B. Tissue Cultures

To observe the dynamic aspects of recovery from metaphase arrest, the study was continued using HeLa and human amnion cells grown in culture. Unless otherwise specified, the concentration of Colcemid or colchicine used was 1×10^{-5} mg/ml.

The general response of the cultures to colchicine and Colcemid is shown in Figs. 2 and 3. Following a lag period of 30 min after Colcemid treatment and 60 min after colchicine treatment, 97 to 100% of the cells entering mitosis were blocked at metaphase (Fig. 2). The accumulation of mitotic figures (Fig. 3) indicated that the drugs did not interfere with preparations for division or with factors involved in the initiation of mitosis. The specific action of low concentrations of colchicine and its derivatives on the mitotic apparatus has been well documented (9).

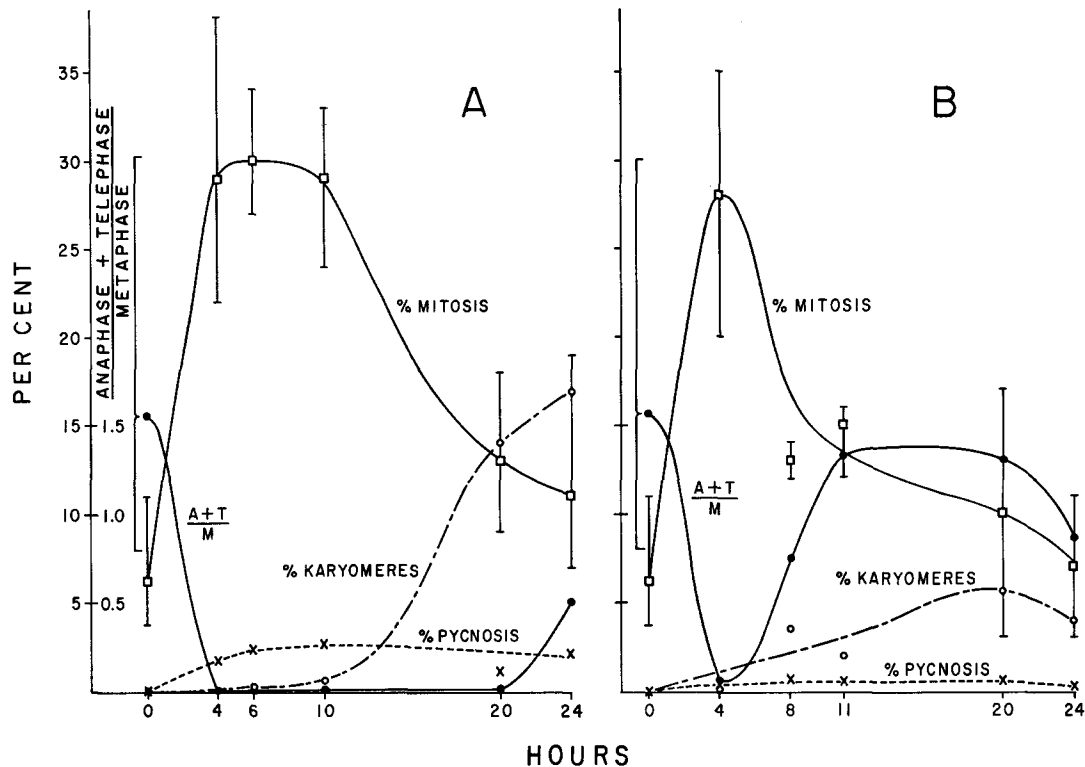


FIGURE 1 The effect of a single injection of Colcemid administered 25 hr after partial hepatectomy. 1, measurements of the mean ratio of anaphase and telophase:metaphase figures ($A+T:M$); 2, the per cent mitosis (plotted to show the range of variation among animal samples); 3, the percentage of cells with three or more karyomeres; and 4, percentages of pycnotic cells are plotted as a function of time after drug administration. Two patterns of recovery are shown. Pattern *A*, the more common response, is characterized by low numbers of anaphase and telophase figures in the 11 to 24-hr samples and the high percentage of cells with three or more karyomeres. Pattern *B*, seen in only an occasional animal, shows that recovery from the blocking effects of the drug starts 8 to 11 hr after drug administration. The 11- to 24-hr samples show increased numbers of anaphase and telophase figures: relatively few cells contain three or more karyomeres.

The following measurements on individual cells were obtained with time-lapse cinemicrography:

1. Mitotic time and intermitotic time prior to the addition of Colcemid (3 cells).
2. Duration of metaphase block during exposure to Colcemid (12 cells).
3. Recovery time after cells were washed free of Colcemid (12 cells).
4. Time from recovery to the next division (3 cells).
5. Mitotic time of cells which came to division after the 6- to 8-hr exposure to Colcemid (15 cells).
6. Intermitotic time of daughters from paragraph 5 (5 cells). The results of these measurements are summarized in Table I.

The untreated cells had mitotic times of 35 to 43 min and intermitotic times of approximately 19 hr (Table I). Cells exposed to Colcemid as they entered mitosis were blocked in metaphase for periods ranging from 10 min to 5.5 hr. These cells were then followed through the period of recovery, defined as the interval between the time the cultures were washed free of the drug to the time of cytokinesis. The recovery period varied from 48 min to over 3.0 hr. Although there was no clear correlation between the period of time the cells were blocked in metaphase and the subsequent recovery time, the three cells blocked for the longest time (3.25, 5.21, and 5.51 hr) recovered within 50 min after being washed free of Colcemid, whereas the recovery time for most other arrested

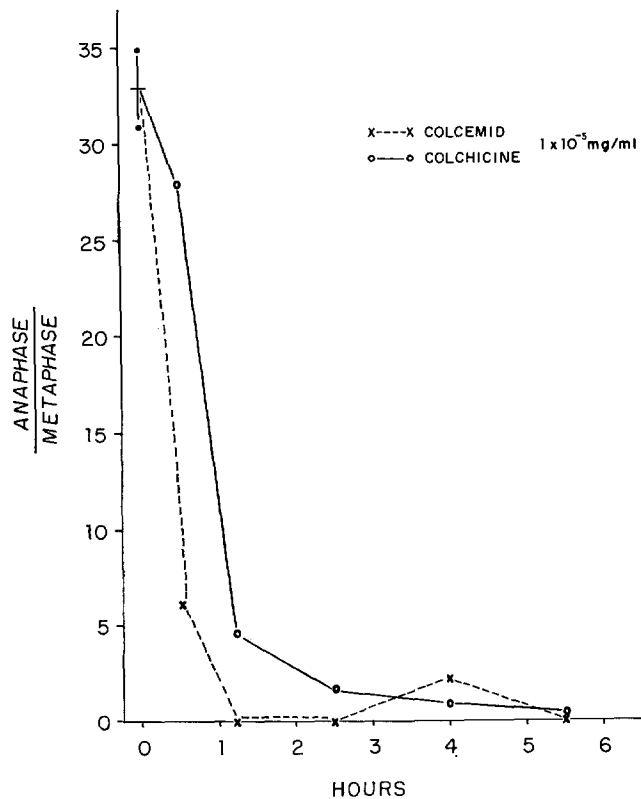


FIGURE 2 The effect of exposure to colchicine and Colcemid, as a function of time, on the ratio of anaphase:metaphase figures in HeLa cultures.

cells was longer, i.e. 72 to 195 min (Table I). 5 to 6 hr appeared to be the upper limit at which cells could remain blocked and still recover by completing mitosis. Cells which were blocked in metaphase in the presence of Colcemid for periods longer than this would suddenly begin violent blebbing and die.

Three daughters of the Colcemid-recovered cells were followed through a subsequent cycle. Each had a normal intermitotic time ranging between 16 and 24 hr (Table I). Cells that remained in interphase during the entire period of exposure to Colcemid showed normal mitotic and intermitotic times in subsequent divisions (Table I).

When cultures were washed free of the drug and replenished with fresh medium, arrested cells were often detached from the glass surface. When such free-floating cells were collected by centrifugation and resuspended in fresh medium, a significant degree of synchrony was noted 25 to 30 hr later as cells progressed through the next mitosis. These cultures contained a small percentage of cells with two or more micronuclei and pycnotic cells, in-

dicating that not all cells recovered from Colcemid arrest by regeneration of a functional spindle.

C. Morphological Aspects of Blockage and Recovery

The size and form of typical control metaphase figures are shown in Fig. 4. Exposure to Colcemid (1×10^{-5} mg/ml) for 2 to 6 hr affected the organization of the mitotic apparatus such that the spindle formed was shrunken, i.e. the distance between the poles of the spindle was reduced (Fig. 5). In many cells a recognizable spindle was formed and maintained, and the chromosomes were fairly well oriented at the equator of the shrunken spindle. Complete dissolution of the spindle occurred in some cells, resulting in a scattering of the chromosomes (star metaphase) (Fig. 6). A clear zone was observed around the reduced or disoriented spindle and was sharply delineated from the denser peripheral zone of the cell by a membranelike structure (Figs. 5 to 7).

The effect on spindle length of exposure to varying concentrations of Colcemid for 2 hr is

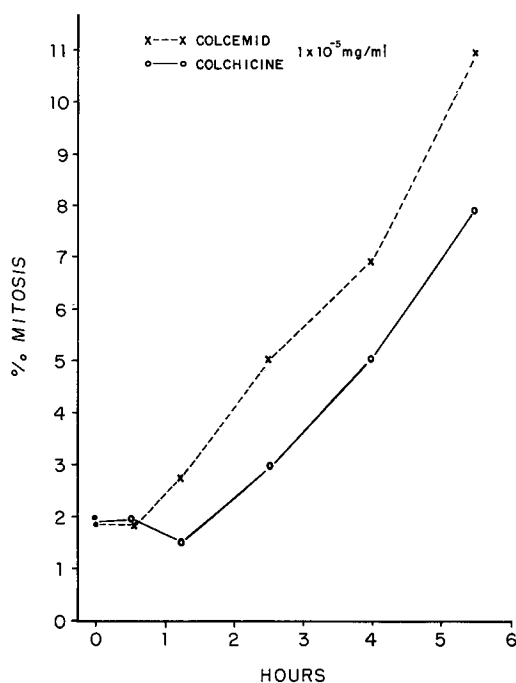


FIGURE 3 The effect of exposure to colchicine and Colcemid, as a function of time, on the percentage of mitotic figures in HeLa cultures.

shown in Table II. The distance between poles of the spindle was measured in cells in which the centrioles were oriented in a single focal plane. Exposure to 10^{-6} mg/ml of Colcemid for 2 hr had no effect on spindle length, but did block cells in metaphase. With increasing concentrations of Colcemid, there was a shift toward shorter spindle lengths (Table II). At concentrations above 5×10^{-5} mg/ml, most cells failed to form a measurable spindle.

Measurements of spindle length in cultures washed free of Colcemid following a 2-hr exposure to the drug are given in Table III. Within 1 hr most of the cells, although still arrested at metaphase, had spindles of normal length. Cells recovering from metaphase arrest were distinguishable from those just entering metaphase by the presence of the clear zone and the membranelike structure which remained even after spindle length was restored (Figs. 8 and 9).

DISCUSSION

Colchicine and its derivatives primarily affect the organization of the mitotic spindle such that cells

entering mitosis are blocked in metaphase. Most cells recover by direct reconstruction to interphase (4, 12, 9, 32). In some instances, the disorienting effects of colchicine on the mitotic spindle are reversed so that cells washed free of the drug reconstitute a functional spindle, i.e. in the developing *Chaetopterus* oocyte (17) and in cleaving eggs of the sea urchin *Mespilia globulus* (24, 25). In the present study, it was shown that the Colcemid-induced blockage may also be reversed in dividing mammalian cells. Arrested cells recover by reconstructing a spindle and dividing to form two daughter cells. With the aid of time-lapse cinemicrography, a quantitative study of spindle formation, the period of metaphase arrest, and patterns of recovery was made.

A. Lag Period

The initial lag (30 min with Colcemid, 1 hr with colchicine) before cells entering mitosis are blocked from continuing on to anaphase is interpreted as reflecting the dose-dependent action of the drug. Apparently, a minimum concentration of the drug must accumulate in the cell before it effectively disrupts spindle structure or maintenance. Studies of grasshopper neuroblasts (12) and *Chaetopterus* oocytes (17) have demonstrated that the effectiveness of the drug is directly proportional to the concentration and time of exposure. The work of Inoué (17) further showed that the action of the drug was not an all-or-nothing type of phenomenon. A progressive breakdown in spindle birefringence occurred with time, dependent on drug concentration. The kinetics of uptake and binding of H^3 -colchicine in human cell cultures has recently been reported by Taylor (46). He calculated that approximately 4 to 8% of the total cell protein may bind with colchicine, and estimated that metaphase block occurred when 3 to 5% of available sites was complexed. The shorter lag period noted in our cultures treated with Colcemid as compared with colchicine suggests that Colcemid may enter the cell at a faster rate and/or is more effective at lower intracellular concentrations.

The pole-to-pole length of the spindle in cells exposed for 2 hr to low drug concentrations shows a unimodal distribution characteristic for each concentration (Table II). This means that the intracellular complexing of the drug probably proceeds at comparable rates during the later part of interphase and during the period of mitotic arrest. This observation complements the work of

TABLE I

Summary of Data Obtained by Time-Lapse Cinemicrography Showing Duration of Mitosis and Intermitotic Times of Individual HeLa Cells Prior to, during, and after Exposure to Colcemid

Time-lapse cinemicrography sequences were made for 24 hr prior to the addition of Colcemid to the perfusion chambers, during the 6-hr period of exposure to Colcemid, and for 24 to 48 hr after removal of the drug.

Before Colcemid		Colcemid block			After Colcemid	
Mitosis	Intermitotic time	Block	Recovery mitosis	Intermitotic time	Mitosis	Intermitotic time
Hr	Hr	Hr	Hr	Hr	Hr	Hr
	19.3	5.21	0.81		0.57	
0.58	18.7	5.51	0.87		0.65	19.3
0.71		1.65	1.21		0.69	17.3
0.74		3.46	0.79		0.58	
		1.53	2.39		0.89	19.8
		1.20	3.25		0.78	
		2.70	1.88	< 16.4 < 18.0	0.57	15.9
		1.03	1.41	24.2	0.93	
		0.33	1.68		0.82	
		0.67	2.73		0.68	23.5
		0.11	1.55		1.18	
		0.41	1.56		0.76	
					1.08	
					1.00	
					0.74	

Taylor (46) who provided direct evidence showing that colchicine entered the cell rapidly during all stages of the cell cycle, and that the level of intracellular binding was a function of the concentration and time of exposure.

B. The Spindle

The mechanisms by which colchicine inactivates the mitotic apparatus have not been unequivocally defined. It has been suggested that the drug reacts directly with the structural elements or molecular precursors of the spindle (27). Inoué (17-19), who described spindle structure as reflecting an equilibrium between oriented and dissociated molecules, suggested that colchicine may antagonize some component in the cell which is involved in the polymerization of spindle proteins, shifting the equilibrium toward dissociation. Certain findings related to colchicine binding and its action on spindle structure focus attention on the dynamic aspects involved in the formation and stability of the spindle. Sauaia and Mazia (41) reported that colchicine and Colcemid had no effect on the structure of the isolated mitotic apparatus of cleaving sea urchin eggs, even at high concentra-

tions. Disorientation occurred only when intact cells were exposed to the drug. In a preliminary report, Taylor (45) noted that H³-colchicine binding appeared to involve more than a simple complexing between the drug and a particular molecular species. In a study of the uptake of H³-colchicine by HeLa cells, cell fractionation showed that 90% of the activity was in the soluble supernatant fraction, primarily bound to large molecules. However, when a cell-free system was treated with the drug, there was no binding in the soluble fraction; some particulate factor was required.

Various types of intermolecular bonds have been implicated in trying to correlate the highly organized tubular fine structure of the spindle with its labile nature. The involvement of thiol reactions has been extensively analyzed, and much has been learned from the successful isolation of the mitotic apparatus in a near natural state (28, 30, 31). Factors that have stabilized the apparatus include (1) pH of 6.2 or lower; (2) presence of divalent cations; (3) presence of a variety of "stabilizing agents" (29, 30, 31, 20). Kane (20) has significantly shown that pH alone was a suffi-

cient stabilizing factor; well preserved mitotic figures were isolated in distilled water at pH 5.5 to 5.6. Further studies by Kane (21) have indicated that a variety of penetrating nonelectrolytes, acting through a nonspecific effect on the solubility properties of spindle proteins, stabilized the mitotic apparatus at higher pH values. Just what factors are involved in the formation and dissolution of the spindle in the intact cell during mitosis, and

how colchicine and other spindle poisons act to impair spindle structure are yet to be resolved. Studies of the interaction of "organizing centers" and localized environmental factors affecting protein solubility, polymerization, and macromolecular aggregation in the formation of structured organelles in general should provide new insights (1-3, 19, 36, 40).

Electron microscopy has revealed that the spindle is composed of microtubules (39, 14, 26). Microtubules have also been identified in most interphase cells (43, 26, 8, 38, 42). They have been described as labile transient structures which may disappear and form anew at different locations in the cell, dependent upon as yet unknown factors (26, 43). When a cell enters into mitosis, microtubules are found only as part of the mitotic apparatus (26, 38). The question arises as to whether colchicine also disorients microtubules of the interphase cell. That it does has been demonstrated by electron micrographs of HeLa cells (38), and indirectly in reovirus-infected tissue culture cells (6, 44). Thus, it appears that colchicine generally disrupts microtubule structure, yet has relatively minor effects on the viability of cells.

C. Membrane Formation

The appearance of a membranous structure enveloping the spindle zone several hours after mitotic arrest was a fairly consistent finding in our cultures (Figs. 6 to 8). The structure develops in the presence of Colcemid and persists into the recovery period. One possible interpretation is that, with time, some aspect of the intracellular milieu affects migration and coalescence of membranous elements. This may, in effect, be similar to the anaphase- and telophase-associated changes that

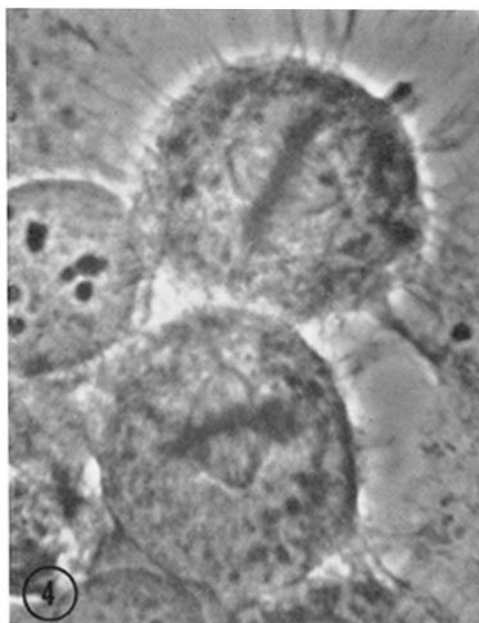
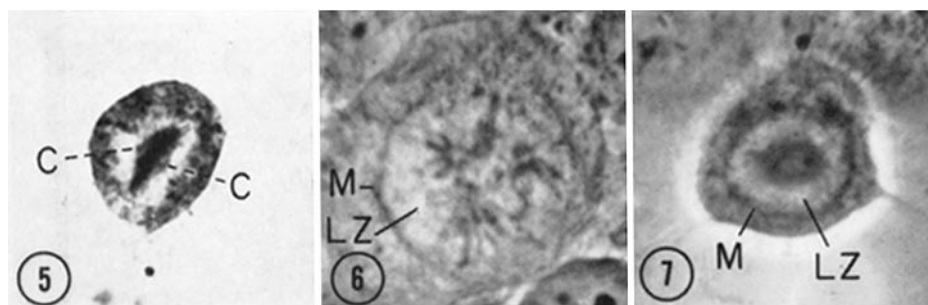


FIGURE 4 Photomicrograph of two normal HeLa cells in metaphase prior to treatment with Colcemid. Cells were fixed in OsO_4 , stained with azure B, and photographed using phase-contrast optics. $\times 1600$.



FIGURES 5 to 7 Photomicrographs of HeLa cells in metaphase arrest after exposure to Colcemid for 2.0 to 2.5 hr. Note the shrunken spindles and the membranous boundary (*M*) around the spindle area. A light zone (*LZ*) is often seen. *C*, centrioles at spindle poles. Fig. 5, $\times 1300$. Figs. 6, 7, $\times 1600$.

TABLE II

Distribution of Spindle Lengths in Ocular Micrometer Units (1 Unit = 0.91 μ) 2 Hr after Treatment with the Indicated Concentration of Colcemid

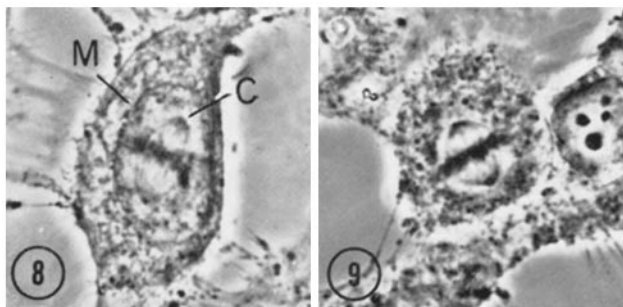
HeLa cells fixed in OsO₄ fumes.

Concentration	Spindle length										
	3.0- 3.9	4.0- 4.9	5.0- 5.9	6.0- 6.9	7.0- 7.9	8.0- 8.9	9.0- 9.9	10.0- 10.9	11.0- 11.9	12.0- 12.9	13.0- 13.9
mg/ml											
Control						1	18	11	6	2	2
1×10^{-6}						1	5	9	3	1	1
3×10^{-6}					4	6	5	3	2		
7×10^{-6}			1	2	9	4	3	1			
1×10^{-5}		1	2	2	10	5					
1.5×10^{-5}	3	10	4	3							

TABLE III

Distribution of Spindle Lengths in Ocular Micrometer Units (1 Unit = 0.91 μ) in HeLa Cells at 2 Hr after Treatment with Colcemid (10^{-5} mg/ml) and during Recovery

Treatment	Spindle length										
	3.0- 3.9	4.0- 4.9	5.0- 5.9	6.0- 6.9	7.0- 7.9	8.0- 8.9	9.0- 9.9	10.0- 10.9	11.0- 11.9	12.0- 12.9	13.0- 13.9
Control				1	2	12	24	10	1	1	
2 hr	3	11	10	1							
2 hr + 1 hr recovery					6	13	4	3	1		
2 hr + 2 hr recovery					1	7	7	8	1	1	



FIGURES 8 and 9 Photomicrographs of human amnion cells exposed to Colcemid for 2 hr, washed, then reincubated in fresh medium for 1 hr prior to fixation. Fig. 8 shows a cell during recovery from metaphase arrest. The spindle length is restored to normal. The membranous boundary (M) around the spindle area is still present. Fig. 9 shows a cell which has entered mitosis after transfer to fresh medium. It cannot be distinguished from metaphase figures seen in control cultures (Fig. 1). C, centrioles at spindle poles. Figs. 8, 9 $\times 1050$.

reportedly involve cisternae of the endoplasmic reticulum in the reformation of the nuclear envelope (34). An electron micrograph of a HeLa cell arrested in metaphase by colchicine (Fig. 5 in reference 38) showed that elements of the endoplasmic reticulum (ER) tended to circumscribe the entire zone of the disoriented apparatus,

whereas in the untreated cell the aggregates of the ER formed at the polar regions (37). In rat thymocytes treated with Colcemid, a coalescence of membranes was described as representing remnants of the nuclear membrane (7). A striking accumulation of concentric membranes was found in arrested HeLa cells exposed to vincristine sul-

fate, a mitotic inhibitor which also disrupts spindle structure (13). The membranes were identified as agranular elements of the endoplasmic reticulum.

D. Recovery

Within approximately 1 hr after cells are washed free of Colcemid, spindle lengths are restored to normal. Some cells then proceed directly to anaphase, while others remain suspended for varying periods of time (Table I). Once anaphase commences, the time from anaphase to cytokinesis is the same in both recovered and untreated cells.

There are instances in nature in which cells remain suspended in metaphase for long periods of time until properly activated; i.e. the developing *Chaetopterus* oocyte may remain in metaphase of the first maturation division for several hours until fertilized (17). As a stage in mitosis, metaphase has been aptly described by Mazia (29) as "... an interruption in the flow of events during which the mitotic apparatus is waiting for something to happen." Just what factors initiate the separation of the daughter chromatids at anaphase are not known. The presence of an intact spindle is apparently not essential, since the separation of daughter chromatids in arrested endosperm cells was observed during *c*-anaphase in the absence of a functional spindle (32).

REFERENCES

1. ABRAHAM, D. and KOFFLER, H., In vitro formation of flagella-like filaments and other structures from flagellin, *J. Mol. Biol.*, 1964, **9**, 168.
2. ANSEVIN, A. T., and LAUFFER, M. A., Polymerization-depolymerization of tobacco mosaic virus protein. I. Kinetics, *Biophysic. J.*, 1963, **3**, 239.
3. ASAKURA, S., EGUCHI, G., and IINO, T., Reconstitution of bacterial flagella *in vitro*, *J. Mol. Biol.*, 1964, **10**, 42.
4. ERUES, A. M., and JACKSON, E. B., Nuclear abnormalities resulting from inhibition of mitosis by colchicine and other substances, *Am. J. Cancer*, 1937, **30**, 504.
5. BUCK, R. C., Lamellae in the spindle of mitotic cells of Walker 256 carcinoma, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 227.
6. DALES, S., Association between the spindle apparatus and reovirus, *Proc. Nat. Acad. Sc.*, 1964, **50**, 268.
7. DE HARVEN, E., and DUSTIN, P., Étude au microscope électronique de la stathmocinèse chez le rat, in *L'Action Antimitotique et Caryoclasique de Substances Chimiques*, Centre National de la Recherche Scientifique, Paris, 1960, 189.
8. DE-THÉ, G., Cytoplasmic microtubules in different animals cells, *J. Cell Biol.*, 1964, **23**, 265.
9. EIGSTI, O. J., and DUSTIN, P., Colchicine in Agriculture, Medicine, Biology, and Chemistry; Ames, Iowa, Iowa State College Press, 1955.
10. EISMAN, P. C., Personal communication and unpublished notes, Microbiology Division, Research Department, Ciba Pharmaceutical Co., Summit, New Jersey.
11. FEINENDEGEN, L. E., and BOND, V. P., Observations on nuclear RNA during mitosis in human cancer cells in culture (HeLa-S₃), studied with tritiated cytidine, *Exp. Cell Research*, 1963, **30**, 393.
12. GAULDEN, M. E., and CARLSON, J. G., Cytological effects of colchicine on the grasshopper

This work was supported in part by grants CA-06784 and CA-04526 from the United States Public Health Service.

Received for publication 21 April 1965.

- neuroblast *in vitro* with special reference to the origin of the spindle, *Exp. Cell Research*, 1951, **2**, 416.
13. GEORGE, P., JOURNEY, L. J., and GOLDSTEIN, M. N., Effect of vincristine on the fine structure of HeLa cells during mitosis, *J. Nat. Cancer Inst.*, 1965, **35**, 355.
 14. HARRIS, P., Some structural and functional aspects of the mitotic apparatus in sea urchin embryos, *J. Cell Biol.*, 1962, **14**, 475.
 15. HARRIS, P., and MAZIA, D., The finer structure of the mitotic apparatus, in *The Interpretation of Ultrastructure*, (R. J. C. Harris, editor), New York, Academic Press Inc., 1962, 279.
 16. HIGGINS, G. M., and ANDERSON, R. M., Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal, *Arch. Path.*, 1931, **12**, 186.
 17. INOUÉ, S., The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle, *Exp. Cell Research, suppl.*, 1952, **2**, 305
 18. INOUÉ, S., Motility of cilia and the mechanism of mitosis, in *Biophysical Science—A Study Program* (J. Conley, editor), New York, John Wiley & Sons, Inc., 1959, 402.
 19. INOUÉ, S., Organization and function of the mitotic spindle, in *Primitive Motile Systems in Cell Biology*, New York, Academic Press Inc., 1964, 549.
 20. KANE, R. E., The mitotic apparatus: isolation by controlled pH, *J. Cell Biol.*, 1962, **12**, 47.
 21. KANE, R. E., The mitotic apparatus. Physical-chemical factors controlling stability, *J. Cell Biol.*, 1965, **25**, 137.
 22. KLEINFELD, R., unpublished data.
 23. KLEINFELD, R. G., and VON HAAM, E., Nucleic acid metabolism in regenerating rat liver using cytidine- H^3 , *Ann. Histochem.*, 1962, **7**, 89.
 24. KOBAYASHI, N., and NAKAMURA, K., The cleavage inhibition and abnormal cleavage of sea-urchin eggs induced by demicolcin. I., *Zool. Mag.*, 1957, **66**, 258.
 25. KOBAYASHI, N., and NAKAMURA, K., The cleavage inhibition and abnormal cleavage of sea-urchin eggs induced by demicolcin. II, *Zool. Mag.*, 1957, **66**, 377.
 26. LEDBETTER, M. C., and PORTER, K. R., A "microtubule" in plant cell fine structure, *J. Cell Biol.*, 1963, **19**, 239.
 27. LEVAN, A., and OSTERGREN, G., The mechanism of C-mitotic action: observations on the naphthalene series, *Hereditas*, 1943, **29**, 381.
 28. MAZIA, D., The central problems of the biochemistry of cell division, in *Biological Structure and Function*, (T. W. Goodwin and O. Lindberg, editors), New York, Academic Press Inc., 1961, **2**, 475.
 29. MAZIA, D., Mitosis and the physiology of cell division, in *The Cell* (J. Brachet and A. E. Mirsky, editors), New York, Academic Press Inc., 1961, **3**, 77.
 30. MAZIA, D., Cell division, in *Sulfur in Proteins*, New York, Academic Press Inc., 1959, 367.
 31. MAZIA, D., MITCHISON, J. M., MEDINA, H., and HARRIS, P., The direct isolation of the mitotic apparatus, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 467.
 32. MOLÈ-BAJER, J., Cine-micrographic analysis of C-mitosis in endosperm, *Chromosoma*, 1958, **9**, 332.
 33. NEBEL, B. R., Cytological observations on colchicine, *Biol. Bull.*, 1937, **73**, 351.
 34. PORTER, K. R., and MACHADO, R. D., Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 167.
 35. PRESCOTT, D. M., and BENDER, M. A., Synthesis of RNA and protein during mitosis in mammalian tissue culture cells, *Exp. Cell Research*, 1962, **26**, 260.
 36. RENAUD, F. L., and SWIFT, H., The development of basal bodies and flagella in *Allomyces arbusculus*, *J. Cell Biol.*, 1964, **23**, 339.
 37. ROBBINS, E., and GONATAS, N. K., The ultrastructure of a mammalian cell during the mitotic cycle, *J. Cell Biol.*, 1964, **21**, 429.
 38. ROBBINS, E., and GONATAS, N. K., Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell, *J. Histochem. and Cytochem.*, 1964, **12**, 704.
 39. ROTH, L. E., and DANIELS, E. W., Electron microscopic studies of mitosis in amebae. II. The giant ameba *Pelomyxa carolinensis*, *J. Cell Biol.*, 1962, **12**, 57.
 40. ROTH, L. E., Motile systems with continuous filaments, in *Primitive Motile Systems in Cell Biology*, (R. D. Allen and N. Kamiya, editors), New York, Academic Press Inc., 1964, 527.
 41. SAUAIA, H., and MAZIA, D., Action of colchicine on the mitotic apparatus, *Path-Biol.*, 1961, **9**, 473.
 42. SANDBORN, E., KOEN, P., MCNABB, J. D., and MOORE, G., Cytoplasmic microtubules in mammalian cells, *J. Ultrastruct. Research*, 1964, **11**, 123.
 43. SLAUTTERBACK, D. B., Cytoplasmic microtubules. I. Hydra, *J. Cell Biol.*, 1963, **18**, 367.
 44. SPENDLOVE, R. S., LENNETTE, E. H., CHIN, J. N., and KNIGHT, C. O., Effect of antimitotic agents

- on intracellular reovirus antigen, *Cancer Research*, 1964, **24**, 1826.
45. TAYLOR, E. W., Studies on the mechanism of inhibition of mitosis by colchicine, *J. Cell Biol.*, 1963, **19**, 70A.
46. TAYLOR, E. W., The mechanism of colchicine inhibition of mitosis. I. Kinetics of inhibition and the binding of H³-colchicine, *J. Cell Biol.*, 1965, **25**, 145.
47. TAYLOR, J. H., Nucleic acid synthesis in relation to the cell division cycle, *Ann. New York Acad. Sc.*, 1960, **90**, 409.
48. WOODARD, J., RASCH, E., and SWIFT, H., Nucleic acid and protein metabolism during the mitotic cycle in *Vicia faba*, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 445.
49. ZIRKLE, R. E., Structure of the mitotic spindle, in Symposium on Molecular Biology, (R. E. Zirkle, editor), University of Chicago Press, 1959, 321.