# ASYNCHRONY OF NUCLEAR DEVELOPMENT IN CYTOCHALASIN-INDUCED MULTINUCLEATE CELLS

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## INTRODUCTION

Synchrony of mitotic nuclei within a multinucleate cell is more of a biological rule than an exception (13). Synchronized division of nuclei in multinucleate cells has been reported in slime molds (10), protozoa (12, 15, 16), insects (18), and mammalian germ and somatic cells (2, 6, 7, 13). The influence of a cytoplasmic humoral factor which controls nuclear division has been supported by observations on the synchrony in cells joined by cytoplasmic bridges (2, 6, 7) and by experiments on nuclear transplantation (5, 8). However, a few rare exceptions to the general rule are known (9, 13, 19).

Cytochalasin B, a mould metabolite from *Helminthosporium dematioideum* isolated by Aldridge et al. (1), causes multinucleate cell formation by suppressing daughter cell separation following an otherwise normal nuclear division (4, 14, 17). In time-lapse cinematographic observations,[1] the mitotic cells round up, chromosomes segregate, and a normal cleavage furrow is seen to develop. The two resulting daughter cells move away from each other, but remain connected by a thin interconnecting bridge often showing a midbody. Subsequently, this connecting bridge fails to break, and the daughter cells reunite and form a large binucleate cell. In some instances, the two resulting nuclei reunite to form a single large nucleus.[1] On continued incubation in the drug-containing medium, this process of nuclear division followed by reunion of the daughter cells is repeated until cells with as many as eight nuclei are formed. As reported earlier by Carter (4), many of these cells have odd numbers of nuclei, suggesting that probably all the nuclei in a multinucleate cell do not divide at the same time. He has suggested that probably a multinucleate cell may enter mitosis when only one of its nuclei is fully prepared while the remaining nuclei may be induced to go through a "pseudomitosis" which closely imitates mitosis but involves no over-all change in the nuclei. Such a cycle could entail chromatin condensation and chromosome formation followed by complete reconstruction of each nucleus in its original form (4).

In short term cultures of human lymphocytes exposed to cytochalasin B, Ridler and Smith (14) reported that all the nuclei in a dividing cell appeared to be at the same stage of division and that

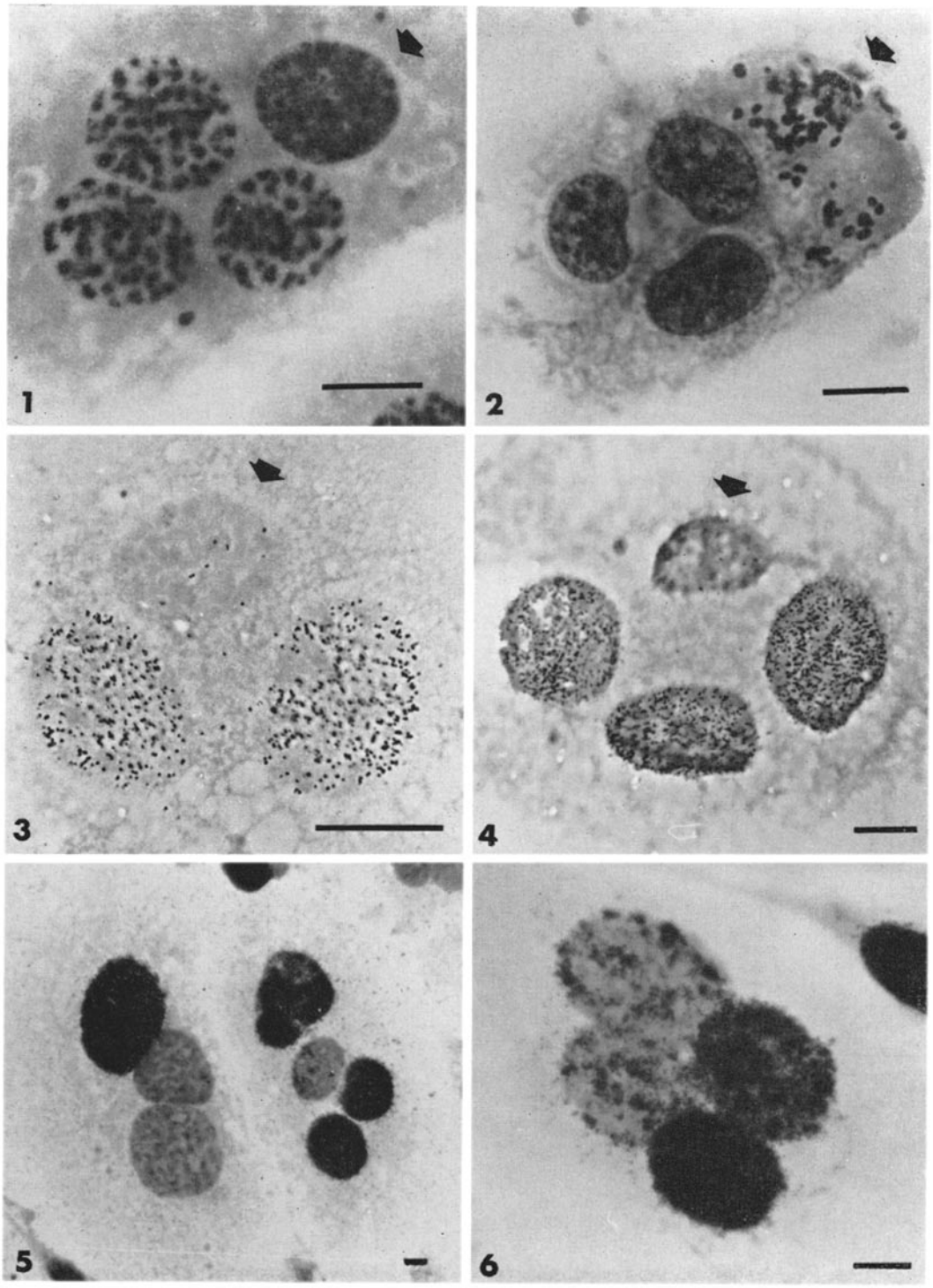
[1] A. Krishan. In preparation.

All the figures are from cultures exposed to cytochalasin B (1 μg/ml) for 72–120 hr. Magnification marker on figures is 10 μm long.

FIGURE 1 Arrow points to an interphase nucleus with diffuse chromatin. The remaining three nuclei of this tetranucleate cell have condensed early prophase chromosomes. × 1,500.

FIGURE 2 A cytochalasin-induced multinucleate cell arrested in mitosis by Velban (0.01 μg/ml, 3 hr). Arrow points to the metaphase chromosomes of one nucleus; the other three nuclei are still in the interphase. × 1,250.

FIGURES 3–6 Radioautographs of thymidine-$^3$H-labeled, cytochalasin-induced multinucleate cells. In Figs. 3 and 4, the arrows indicate nuclei with only a few reduced silver grains; these nuclei show relative nonincorporation of thymidine-$^3$H label compared to the remaining nuclei in the same cell. Fig. 5 shows two multinucleate cells with heavy and lightly labeled nuclei. In Fig. 6, four nuclei of a multinucleate cell show different amounts of radioactive label uptake as indicated by the number of reduced silver grains on their surface. Fig. 3, × 1,875; Fig. 4, × 875; Fig. 5, × 300; and Fig. 6, × 850.


1
2
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none of the cells had a mitotic figure as well as an interphase nucleus.

In the present study, we have tried to explore, with the help of thymidine-$^3$H radioautography and Velban-(vinblastine sulfate, Eli Lilly, Indianapolis) induced mitotic arrest, the asynchrony of nuclear development, and mitosis in cells exposed to cytochalasin B.

## MATERIALS AND METHODS

Earle's L-929 fibroblasts were grown in Leighton tubes on medium 199 supplemented with 10% fetal calf serum, penicillin (100 I.U./ml) and streptomycin (100 μg/ml). After 42 hr, the old medium was replaced with fresh, prewarmed medium containing 1 μg/ml of cytochalasin B. Following the suggestion of Carter (4), stock solution of cytochalasin B (1 mg/ml) was made in dimethylsulfoxide and stored at 4°C.

Cultures exposed to cytochalasin for 72–120 hr were labeled for 30 min–24 hr with fresh medium containing thymidine-$^3$H (5 μc/ml). Following the labeling, the cultures were washed three to four times in prewarmed Hanks' balanced salt solution, and fixed in Carnoy's 1:3 acetic:alcohol. Coverslips from these preparations were coated with Ilford K-5 liquid emulsion, dried, and stored at 4°C. After 15 days of exposure time, preparations were developed in Kodak D-19 at 22°C for 2 min, washed in distilled water, and fixed for 3 min. In a second series of experiments, Leighton tube cultures exposed to cytochalasin B for 120 hr were incubated in fresh medium containing Velban (0.01 μg/ml) for 3 hr to arrest mitotic cells. Following this treatment, cultures were washed, reincubated in fresh medium, and fixed after varying lengths of time from 3 to 10 hr. All preparations were stained with carbol fuchsin (3) and 1% fast green FCF.

## OBSERVATIONS AND COMMENTS

Cultures incubated with cytochalasin B show progressive accumulation of cells with multiple nuclei. In earlier stages, e.g. 76 hr, nearly all multinucleate cells have even numbers (two or four) of nuclei. On further incubation in the cytochalasin-containing medium, an increasing number of cells show odd numbers of nuclei, till by the 5th day there are nearly equal numbers of cells with odd or even numbers of nuclei.

In some of the multinucleate cells, the nuclei appear to be at varying stages of development, as judged by their chromatin condensation. Fig. 1 shows a tetranucleate cell from a culture exposed to cytochalasin B for 120 hr. As seen in this figure, three of the four nuclei have discrete, condensed prophase chromosomes enveloped by the intact nuclear membrane. The fourth nucleus (arrow) is definitely in a different stage of development, as indicated by the diffuse appearance of chromatin in this nucleus. In cultures arrested in mitosis by Velban, this asynchrony of nuclear development becomes all the more apparent as seen in Fig. 2. The arrow in this figure points to the scattered chromosomes in the cytoplasm of a cell (presumably from a single nucleus); the three other intact interphase nuclei share the common cytoplasm with the scattered chromosomes.

Pulse-labeling with thymidine-$^3$H for 30 min followed by incubation in cold medium confirms the asynchrony of nuclear development in some of these cells. Figs. 3 and 4 show multinucleate cells with three and four nuclei, respectively. Arrows point to the nuclei which did not take up the label and thus do not show any appreciable number of developed silver grains on their surface.

Figs. 5 and 6 show the difference in the uptake of the thymidine-$^3$H by the nuclei of cytochalasin-induced multinucleate cells exposed to the labeled precursor for 24 hr. In Fig. 5, the cell on the left has one labeled and two unlabeled nuclei, while the neighboring cell has two heavily labeled nuclei, one partially labeled nucleus, and one lightly labeled nucleus. In Fig. 6, a similar pattern of differential labeling of the nuclei is seen. In culture labeled with thymidine-$^3$H, approximately 25% of the multinucleate cells show asynchronous uptake of the radioactive label.

These observations, which are based on three different experimental designs, i.e. cells from normal cultures, are exposed to cytochalasin and then either arrested in mitosis or labeled with thymidine-$^3$H, confirm the observations of Carter (4) who first suggested that some of the nuclei in cytochalasin-induced multinucleate cells may be out of synchrony although sharing a common cytoplasm with other nuclei. He has based his conclusion on the presence of odd numbers of nuclei in some of the cytochalasin-induced multinucleate cells.

In some earlier observations (11),[1] large groups of centrioles (often as many as ten) have been reported in cultures treated with cytochalasin. Further experiments are in progress, and it will be interesting to see whether all or only one pair of these centrioles take part in the formation of the spindle, when only one of the nuclei enters mitosis and the others remain in the interphase stage.

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## REFERENCES

1. ALDRIDGE, D. C., J. J. ARMSTRONG, R. N. SPEAKE, and W. B. TURNER. 1967. *Chem. Commun.* **1**:26.
2. BURGOS, M. H., and D. W. FAWCETT. 1955. *J. Biophys. Biochem. Cytol.* **1**:287.
3. CARR, D. H., and J. E. WALKER. 1961. *Stain Technol.* **36**:233.
4. CARTER, S. B. 1967. *Nature.* **213:** 261.
5. DE TERRA, N. 1960. *Exp. Cell Res.* **21**:34.
6. FAWCETT, D. W., S. ITO, and D. SLAUTTERBACK. 1959. *J. Biophys. Biochem. Cytol.* **5**:453.
7. FAWCETT, D. W. 1961. *Exp. Cell Res. Suppl.* **8**:174.
8. GOLDSTEIN, L., and D. M. PRESCOTT. 1966. *In* The Control of Nuclear Activity. L. Goldstein, editor. Prentice Hall, New Jersey. 3.
9. HAQUE, A. 1953. *Heredity.* **7**:429.
10. HOWARD, F. L. 1932. *Ann. Bot.* **46**:461.
11. KRISHAN, A., and N. BOHONOS. 1968. Proceedings of the Electron Microscope Society of America, 26th Meeting. C. J. Arceneaux, editor. Claitor Publishing Division, Baton Rouge. 122.
12. KUDO, R. R 1947. *J. Morphol.* **80**:93.
13. MAZIA, D. 1961. *In* The Cell. J. Brachet and A. E. Mirsky, editor. Academic Press Inc., New York and London. **3**:388.
14. RIDLER, M. A. C., and G. F SMITH. 1968. *J. Cell Sci.* **3**:595.
15. SESHACHAR, B. R., and P. B. PADMAVATI. 1956. *Curr. Sci.* **25**:281.
16. SHORT, R. B. 1946. *Biol. Bull.* **90**:8.
17. SMITH, G. F., M. A C. RIDLER, and J. A. FAUNCH. 1967. *Nature.* **216**:1134.
18. SONNENBLICK, B. P. 1950. *In* Biology of Drosophila. M. Demerec, editor. Wiley, New York. 62.
19. STUBBLEFIELD, E. 1964. *In* Cytogenetics of Cells in Culture. Symposia of the International Society for Cell Biology. R. J. C. Harris, editor. Academic Press Inc., New York and London. **3**.