PROTEIN SYNTHESIS DURING REESTABLISHMENT OF MITOSIS AFTER TREATMENT WITH COLCHICINE AND COLCEMID

E. V. Kazan'ev and O. M. Zapara

UDC 615.277.3.015.44:612.014.3:612.6

Autoradiographic investigations with leucine-^{3}H showed that after the action of colchicine on a culture of Chinese hamster cells recovery of the normal course of mitosis is accompanied by marked activation of protein synthesis above its initial level. This increase in protein synthesis precedes complete normalization of the mitotic regime of the cell culture. After treatment with colcemid the recovery period is characterized only by stabilization of protein synthesis at the initial control level.

KEY WORDS: protein synthesis; mitosis; colchicine; colcemid.

Recent investigations have shown that the normal course of mitosis can be restored after stathmokinetic block [2, 6, 7, 9]. Experiments in which inhibitors of protein synthesis were administered after stathmokinetic block by colchicine [1, 3, 8] suggested that mitotic microtubules are restored either by repolymerization of existing tubulins or by the formation of new ones.

To test these hypotheses an autoradiographic study of protein synthesis was made during normalization of mitosis after exposure to colchicine and colcemid.

EXPERIMENTAL METHOD

Experiments were carried out on cultures of Chinese hamster fibroblast-like cells (clone 237). The cells were seeded in penicillin flasks in a concentration of 150,000 cells/ml and grown in Eagle's medium with 10% bovine serum. Either colchicine (1 μg/ml) or colcemid (0.3 μg/ml) was added to the flasks 24 h after seeding. The cells were incubated for 30 min with colchicine and 2 h with colcemid. After treatment with the alkaloid the slides with the cultures were washed with Hanks's solution and transferred to fresh culture medium. Leucine-^{3}H (10 μCi/ml, specific activity 2.5 mCi/mole) was added for 10 min before treatment with the mitotic poisons (control) and again immediately after removal of the alkaloids, and then hourly for 6-8 h of the experiment. Material was fixed in 96% alcohol and processed for light-optical autoradiography in the usual way. The intensity of protein synthesis was judged from the number of tracks above metaphase cells. The intensity of incorporation of the isotope into intact metaphse cells served as the control. Meanwhile, the mitotic index was determined for each preparation. All experiments were repeated four times. Statistical analysis of the results was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

After exposure to both alkaloids the usual stathmokinetic response was observed and was accompanied by a sharp increase in the number of metaphases (70-80%) and total disappearance of anaphases and telophases (Figs. 1 and 2). After removal of the alkaloids the number of metaphases continued to rise for 1-2 h, after which it gradually fell. Changes in the mitotic regime after treatment with colchicine and colcemid were similar in character, the only difference being that after colcemid anaphases and telophases (escape from the block) began to appear after 2 h and reached the control level by 6 h of the recovery period, whereas after colchicine escape from the block did not begin until after 4 h.

Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 588-589, May, 1977. Original article submitted September 6, 1976.

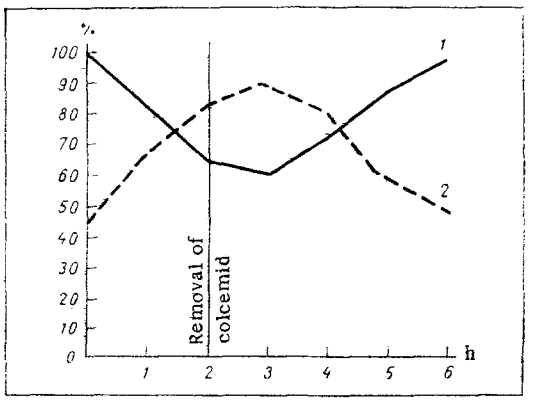

Fig. 1

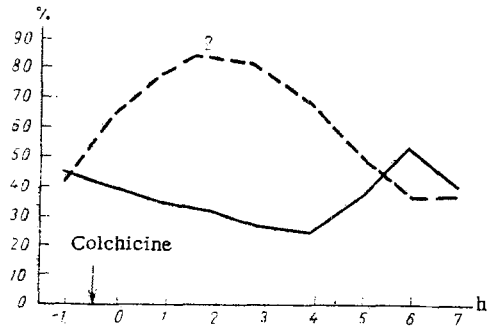

Fig. 2

Fig. 1. Changes in intensity of incorporation of leucine-^{3}H after treatment with and removal of colcemid. 1) Incorporation of leucine-^{3}H into metaphase cells; 2) percentage of metaphase. Abscissa, duration of experiment (in h); ordinate, number of tracks (in %).

Fig. 2. Changes in intensity of incorporation of leucine-^{3}H into metaphase cells after treatment with colchicine. Legend as in Fig. 1.

The autoradiographic investigations showed that the action of colcemid led to an appreciable decrease in leucine-^{3}H incorporation. The number of tracks above the metaphase cells continued to fall for 1 h after removal of the alkaloid and started to rise gradually only with the appearance of anaphases and telophases, to reach the initial levels at the time of normalization of the mitotic regime (Fig. 1). Changes in the intensity of protein synthesis after treatment with colchicine were rather different (Fig. 2). Incorporation of leucine-^{3}H decreased after incubation of the cells for 30 min with the alkaloid. After removal of the colchicine the number of tracks above the metaphase cells continued to fall for a further 4 h. At the beginning of the recovery period the quantity of isotope taken up increased appreciably, and by 6-7 h it reached a maximum, which was higher than the intensity of incorporation of leucine into intact metaphase cells. This peak of additional protein synthesis preceded complete recovery of the normal mitotic regime and, to judge from the results of electron-microscopic investigations [1], it corresponded to the period of formation of microtubules and of reconstruction of the mitotic apparatus.

The results thus show that, despite the general character of the change in the mitotic regime after treatment with colchicine and colcemid, periods of recovery of the course of mitosis in the two groups of experiments differed significantly. Whereas the recovery period after colcemid was accompanied by stabilization of protein synthesis only, normalization of the mitotic regime after exposure to colchicine was accompanied by additional protein synthesis. The results of these investigations, together with data in the literature [5, 7], suggest that after exposure to colcemid the restoration of mitosis probably takes place through repolymerization of the tubulins of the microtubules. Recovery of the mitotic apparatus after the blocking of mitosis by colchicine requires additional protein synthesis and evidently takes place through the formation of new microtubules. This hypothesis is in agreement with observations according to which the blocking of synthesis after treatment with colchicine delays both normalization of the mitotic regime of Chinese hamster cell cultures [1] and restoration of the microtubules of the cilia of Tetrahymena [8] and the flagella of Ochromonas danica [3].

LITERATURE CITED

1. I. A. Alov, M. E. Aspiz, and N. A. Starosvetskaya, Zh. Obshch. Biol., 35, No. 6, 895 (1974).
2. I. F. Kurilo, Byull. Éksp. Biol. Med., No. 3, 97 (1973).
3. G. Bouck and D. Brown, J. Cell Biol., 47, 22 (1970).
4. A. Forer, Chromosoma, 19, 44 (1966).
5. S. Inoué, in: Primitive Motile Systems in Cell Biology (ed. by R. D. Allen and N. Kamiya), Academic Press, New York (1965), pp. 549-598.
6. S. Malawista, H. Sato, and K. Bensch, Science, 160, 770 (1968).
7. A. Murin, Biologia (Bratislava), 20, 569 (1965).
8. J. Rosenbaum and K. Carlson, J. Cell Biol., 40, 415 (1969).
9. E. Stubblefield, in: Cytogenetics of Cells in Culture (ed. by R. J. C. Harris), Academic Press, New York (1964), pp. 223-248.