EFFECT OF COLCHICINE ON MITOTIC BEHAVIOR OF FIBROBLAST-LIKE CELLS OF LINE 237 OF CHINESE HAMSTERS

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Changes in the mitotic activity of line 237 Chinese hamster cells during the development of colchicine mitosis and liberation of the cells from the metaphase block after removal of the colchicine were studied. To begin with, c-mitoses with partial disturbance of the division spindle (star metaphases) appeared, to be followed by mitoses with scattering of the chromosomes (total disorganization of the mitotic apparatus). The metaphase block appeared 2 h after the beginning of action of the alkaloid. Exposure of the cells for 30 min in colchicine solution followed by their transfer to medium without the alkaloid also led to complete metaphase block after 2 h. The normal mitotic activity was restored only 5-6 h after removal of the colchicine. In some experiments the appearance of a second stathmokinetic wave lasting not less than 10 h was observed.

Only a few investigations have been made of the stathmokinetic effect of colchicine on mammalian cells in vitro [4, 5]. In the writer's laboratory the effect of colcemid on dividing Chinese hamster cells of line 451 has been studied [3]. Colchicine is known to have a more toxic action on the cells than colcemid [6].

To compare the action of these alkaloids the order of development of the stathmokinetic effect of colchicine and the possibility of liberation of the cells from the metaphase block after removal of the alkaloid from the medium were studied.

EXPERIMENTAL METHOD

Cells from Chinese hamsters of line 237 were used as the test object. The method of cultivating the cells, the experimental technique, and the principle used to assess the action of colchicine were similar to those described previously [1-3].

EXPERIMENTAL RESULTS

After the action of colchicine (1 μg/ml) for 10 min the number of pathological mitoses was increased (Fig. 1), mainly on account of the appearance of star-metaphases ($P < 0.001$). A complete block of mitosis in metaphase took place after exposure of the cells for 2 h in the solution of the alkaloid. By this time the mitotic index (MI) was doubled and the predominant form of pathology of mitosis was metaphase with scattering of the chromosomes and cluster metaphases ($P < 0.001$). The number of cells with chromosome deletions in metakinesis and the number of tripolar metaphases did not exceed the control level.

During the development of the colchicine effect (with this dose and duration of exposure to colchicine), by contrast with the action of colcemid, no successive change from one form of pathology of mitosis to another took place but typical c-mitoses appeared immediately, coupled with more or less complete disorganization of the mitotic apparatus (star metaphases, scattering of the chromosomes).

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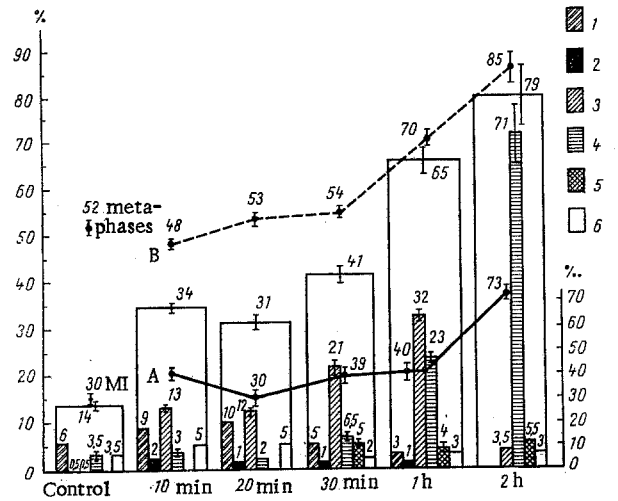

Fig. 1. Mitotic activity of line 237 Chinese hamster cells after exposure to colchicine (1 μg/ml): A) mitotic index; B) number of metaphases. Broad unshaded columns represent pathological mitoses (in %); narrow columns: 1) chromosome deletions in metakinesis, 2) tripolar metaphases, 3) star metaphases, 4) scattering of supercoiled chromosomes, 5) sphere-metaphases and compact coil-metaphases, 6) other forms of pathology of mitosis, combined into one group. Abscissa, duration of action of colchicine; ordinate, mitotic index (in ‰), relative number of metaphases and of pathological mitoses (in ‰).

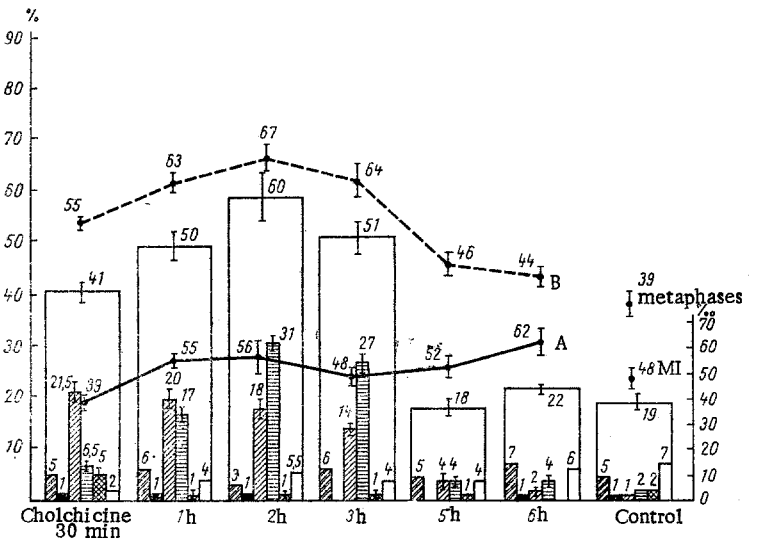

Fig. 2. Mitotic activity of line 237 Chinese hamster cells during recovery of cell division (after exposure for 30 min to colchicine in a dose of 1 μg/ml). Legend as in Fig. 1.

To study whether normal cell division could be restored, after exposure of the cultures for 30 min to colchicine solution they were washed three times and transferred to medium without colchicine, and the mitotic activity was studied at various times of fixation (hourly until 10 h; Fig. 2). Exposure to colchicine for 30 min led to a twofold increase in the number of pathological mitoses, represented by star metaphases and metaphases with scattering of the chromosomes. In the first 2-3 h after removal of the colchicine the mitotic index, number of metaphases, and number of pathological mitoses continued to increase. After 5-6 h the number of metaphases and pathological forms of mitosis showed a decrease almost down to the control level. In some cases (2 of 3 experiments), however, after restoration of the normal indices of mitotic activity 5-6 h after removal of the colchicine, a second wave of the stathmokinetic effect began and it lasted not less than 10 h (the maximal period of observation). Mitotic index, the number of metaphases, and the number of c-mitoses with scattered or compactly arranged chromosomes increased again.

Comparison of the development of the stathmokinetic effect after exposure to colcemid [3] and to colchicine thus shows certain essential differences in the action of the two alkaloids, although the experiments were carried out on different sublines (451 and 237) of Chinese hamster cells.

The stathmokinetic effect of colchicine began to appear only in a concentration of 1 μg/ml, compared with colcemid in a concentration of 0.03 μg/ml. However, the first appreciable disturbances of mitosis occurred after treatment for 10 min with colchicine, whereas under the influence of colcemid statistically significant changes appeared only after 20 min.

A stathmokinetic effect of colcemid was still observed 45-60 min after its removal (exposure of 2 h), whereas the duration of the after-effect was considerably longer after removal from colchicine solution (exposure for 30 min), for it continued up to 5 h or more.

Restoration of normal mitotic activity was observed 1.5-2 h after removal of the colcemid but not until 5-6 h after removal of the colchicine; a second wave of stathmokinetic effect can probably begin after the period of recovery.

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