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MECHANISM OF REVERSIBILITY OF COLCEMID-INDUCED COLCHICINE MITOSIS

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Restoration of the normal course of mitosis after its blocking by colchicine takes place through additional protein synthesis – through the formation of new microtubules of the division spindle. A study of the reversibility of the stathmokinetic reaction to colcemid shows that blocking of protein synthesis by puromycin has little or no effect on the rate of restoration of the normal mitotic regime, whereas copper ions retard this process. It can be concluded from these facts that the restoration of the colcemid-induced C mitosis takes place chiefly through the repolymerization of tubulins. The predominance of the formation of new microtubules or repolymerization during the restoration of the microtubules is evidently determined both by the degree of their destruction and by the character of binding of the subunits of the tubular protein pool with the harmful agent.

KEY WORDS: Mitosis; microtubules; tubulins; stathmokinetic reaction; colcemid.

In a previous communication [2] we examined the mechanism of reversibility of the colchicine mitosis (C mitosis) induced by colchicine. Electron-microscopic and autoradiographic investigations showed that for the normal course of mitosis to be restored after treatment with colchicine additional protein synthesis is required [1, 2]. The peak of intensity of protein synthesis preceded complete restoration of the normal mitotic regime and corresponded in time to the period of formation of microtubules of the division spindle and reconstruction of the mitotic apparatus. It was not made clear whether the formation of new microtubules is the universal mechanism of reversibility of the C mitosis or whether this process can also take place through the repolymerization of microtubules from the tubulin pool [3, 8, 12, 13].

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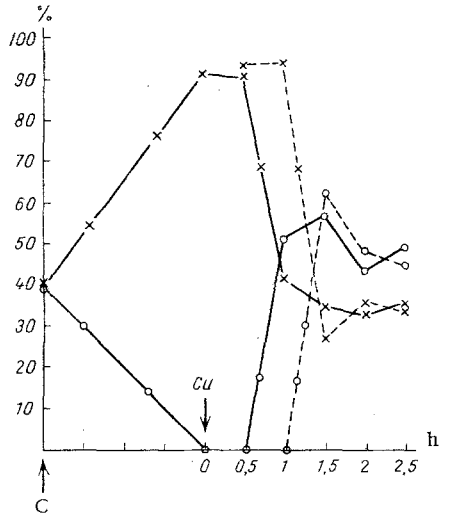

Fig. 1. Indices of mitotic regime during reversibility of C mitosis induced by colcemid (continuous lines) and after additional exposure to Cu^{2+} ions (broken lines). Lines with crosses – metaphases; lines with dots – anaphases + telophases; C) addition of colcemid (control – background); O) rinsing out colcemid and adding Cu^{2+}. Ordinate, relative number of metaphases, anaphases, and telophases (in %); Abscissa, time (in h).

To test this hypothesis the reversibility of the stathmokinetic reaction induced by other factors blocking mitosis in metaphase was studied. In the present investigation the mechanisms of reversibility of the C mitosis induced by a colchicine derivative, Cocemid (N-diacetyl-N-methylcolchicine), were studied.

EXPERIMENTAL METHOD

Experiments were carried out on a monlayer culture of fibroblast-like cells of the Chinese hamster, strain 237. The cells were grown in Eagle's medium with 10% bovine serum. The seeding density was about 100,000 cells/ml. After growth of the cells for 48 h colcemid (0.03 μg/ml) was added to the medium. After exposure to the alkaloid for 2 h, coverslips with the cultures were washed three times with Hanks' solution and transferred to fresh medium. Immediately after rinsing off the colcemid or 30 min later, puromycin (10 μg/ml) was added to the culture medium in the vessels containing the cultures. At various times after rinsing out the colcemid the indices of the mitotic regime (mitotic index, relative percentage of cells in the various stages of mitosis) were determined in the control (cells after treatment with colcemid only) and experimental series (treatment with colcemid after blocking protein synthesis with puromycin). These experiments were carried out in five repetitions. In another series of experiments (four repetitions), after rinsing off the the colcemid, Cu^{2+} ions (anhydrous $CuSO_4$ in a final concentration of 10^{-5} M), which are known [8, 11] to inhibit tubulin repolymerization, were added to the culture medium.

EXPERIMENTAL RESULTS

After exposure to colcemid for 2 h the percentage of cells at the metaphase stage increased by 1.7-2 times, with the result that the mitotic index almost doubled. The final stages of mitosis (anaphase and telophase) were completely absent until the alkaloid was rinsed out. Metaphases continued to accumulate for 1 h after rinsing out (the aftereffect), but then the mitotic regime began to return to normal: the number of metaphases decreased and the number of anaphases and telophases increased. Normalization of the mitotic regime was complete 2-2.5 h after rinsing out the alkaloid. Blocking protein synthesis with puromycin had hardly any effect on the rate of restoration of the normal mitotic regime, by contrast with the reversibility of the C mitosis induced by colchicine, when puromycin delayed the normalization of the mitotic regime as a rule by 14 h (in some experiments the stathmokinetic effect lasted for 20-22 h). Statistically significant differences between the control and experimental series during reversibility of C mitosis induced by colcemid (slight delay in the rate of recovery) was observed in only one of five experiments. In parallel experiments involving incorporation of

leucine-^{3}H (V. V. Kazan'ev) no additional peak of intensity of label uptake was observed during restoration of colcemid mitosis, by contrast with that observed in colchicine-induced C mitosis.

The results suggested that the formation of new microtubules occupies an unimportant place in the reversibility of the colcemid-induced C mitosis. Presumably the reversibility of the stathmokinetic reaction in those experiments was due chiefly to repolymerization of microtubules from tubulins of the precursor pool. Experiments in which copper ions were added confirmed this possibility. In the presence of Cu^{2+} normalization of the mitotic regime after the blocking of mitosis by colcemid was delayed by 30-60 min (Fig. 1).

The mechanisms of restoration of microtubules after treatment even with such closely related compounds as colchicine and colcemid are thus different. In the first case the microtubules were restored mainly through new formation, but in the second mainly through repolymerization.

The difference in the mechanisms of reversibility of the stathmokinetic reaction induced by colchicine and colcemid is probably attributable to differences in the action of these alkaloids of tubulins of the reserve stock. The need for additional protein synthesis and predominance of the formation of new microtubules after the stathmokinetic action of colchicine can be understood [2] on the grounds that this alkaloid binds the tubulins of the reserve stock. Colchicine, by forming stable bonds with subunits of tubulins, inactivates them and thereby depletes their reserve stock. The special features of reversibility of the colcemid effect are evidently attributable to the fact that, unlike colchicine, it binds tubulins much less strongly and forms weaker bonds with them [7]. These results, together with observation published previously [1, 2] suggest that repair of the microtubular systems of the mitotic apparatus, when damaged by stathmokinetic poisons, can take place in various ways: either by their formation de novo (binding of the tubulins of the reserve stock by colchicine) or predominantly through repolymerization of the tubulins of the precursor pool (colcemid). It can tentatively be suggested that these methods of repair also extend to other systems of microtubules. For instance, repair of the microtubules of the cilia of the infusorian Tetrahymena after x-ray irradiation take place through repolymerization [9], whereas their repair after treatment with colchicine requires additional protein synthesis [10]. After treatment with colchicine, inhibition of protein synthesis prevented repair of the cytoplasmic microtubules in the flagellate Ochromonas, but after their destruction by an increased hydrostatic pressure, blocking protein synthesis had no effect on rapair of the microtubules [4-6].

The predominance of their formation de novo or of repolymerization during repair of microtubules is probably determined both by the degree of their destruction and by the character of binding of the harmful agent with subunits of the tubular protein pool – the chief source for restoration of the microtubules.

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