COLCHICINE MITOSIS AND ITS REVERSIBILITY

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Changes in the mitotic indices of cells of Chinese hamsters of line 451 were studied during colchicine mitosis and recovery of the cells from the metaphase block. During development of the c-mitosis under the influence of colcemid there was a regular succession of pathological forms of mitosis connected with partial (delay of the chromosomes in metakinesis, three-group metaphases, "star" metaphases) or complete disorganization (dispersal of the chromosomes, "ball" metaphases, clumped metaphases) of the mitotic apparatus. The effect is directly proportional to the dose and time of action of colcemid. After removal of the alkaloid, the normal course of mitosis is restored, and this also takes place through the appearance of pathological forms of mitosis such as delay of the chromosomes in metakinesis and three-group metaphases.

The colchicine mitosis (c-mitosis) is one of the most widespread forms of mitosis pathology. The antimitotic effect of colchicine and its derivatives was described many years ago [4, 6, 7, 12, 14], but our information on the successive stages of development of the colchicine effect and the features distinguishing the pathology of mitosis arising after exposure to antimitotic poisons is still extremely limited. The question of the reversibility of the c-mitosis and the possibility of recovery of the normal course of mitosis after removal of the colchicine has only begun to be studied in recent years [10, 11, 13, 17, 18]. With these facts in mind it was decided to study the successive stages of development of the colchicine mitosis and to examine whether this type of pathology of mitosis in mammalian cells is reversible.

For this purpose changes in the indices of mitosis were studied during the development of c-mitosis and during recovery of the cells from the colchicine block.

EXPERIMENTAL METHOD

The colchicine derivative colcemid (n-deacetyl-n-methylcolchicine), which is more effective and less toxic than colchicine itself [18, 19], was used for the experiments. Cell cultures from Chinese hamsters of subline 451, isolated from line Bll dii FAF-28 were used. The cells were grown by the usual method. The seeding density was 100,000 cells per ml. A 48-h culture was used in the experiments. The final concentration of colcemid was 0.03 μg/ml. Cultures incubated under the same conditions but without colcemid, but with change of the growth medium at the same times as the experimental cultures, were used as the control.

In experiments to study the recovery of cell division after exposure for 2 h to colcemid solution the cultures were washed three times in Hanks' solution and transferred to growth medium (without colcemid), in which they were cultivated for between 5 min and 3 h.

After fixation and staining with Carazzi's hematoxylin, the number of mitoses per 2000 cells was counted in each preparation. The effect of colcemid was evaluated from the following indices: mitotic activity (per thousand cells), ratio between individual phases of mitosis, and the total number of pathological

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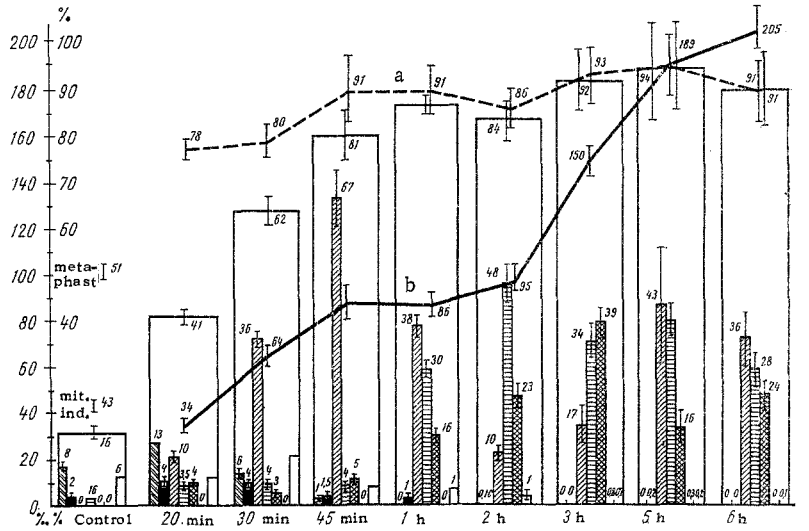

Fig. 1. Mitotic activity of fibroblasts from Chinese hamster of line 451 treated with colcemid (0.03 μg/ml). Abscissa, time of treatment with colcemid; ordinate, mitotic index (in %), relative number of metaphases and pathological mitoses (in %); a) number of metaphases; b) mitotic index; wide unshaded columns show pathological mitoses; narrow columns: shaded with oblique double lines – delay of chromosomes in metakinesis; shaded black – three-group metaphases; shaded with single oblique lines – "star" metaphases; shaded with horizontal lines – dispersal of supercoiled chromosomes; cross-hatched – "ball" and compact coil-metaphases; unshaded narrow columns – clumped metaphases, other forms of pathology of mitosis, combined into one group.

mitoses and the number of the various forms relative to the total number of mitoses. The pathology of mitosis was classified by reference to Alov's scheme [1]. The Fisher–Student method was used for statistical analysis of the experimental results.

EXPERIMENTAL RESULTS

Investigation of the development of c-mitosis showed that treatment with colcemid led to an increase in the mitotic index connected with delay of division (or even complete block) at the metaphase stage and with an increase in the number of the various forms of pathology of mitosis. During the first 45 min the mitotic index increased by 2-2.5 times above the control (Fig. 1), after which there was no significant change in the number of dividing cells until 2 h. Between 2 h and 5-6 h after the addition of colcemid the mitotic index increased by 5-6 times over the control (from 35-45 to 200-240 ‰; $P < 0.001$). The increase in mitotic index was a linear function of time, as other investigators also have observed [11, 18]. It is interesting to note that the mitotic index continued to rise for a further 45-60 min after removal of the colcemid (Fig. 2).

The relative number of metaphases reached a maximum (90-95%) after exposure to colcemid for 30-45 min and it remained at this level throughout the subsequent period of observation (Fig. 1). The number of prophases during the first hour of exposure of the cells to the colcemid solution varied from 5 to 20%, after which it was stabilized at the 5-10% level. Anaphases and telophases disappeared completely after exposure to colcemid for 30 min, indicating a complete blocking of division at the metaphase stage.

A distinct colchicine effect began to appear after exposure to colcemid in a dose 0.03 μg/ml for 20 min. The very slight increase in the number of dividing cells was accompanied by an increase in the number of metaphases and a sharp increase in the number of pathological mitoses ($P < 0.001$). The pathological forms included metaphases with delayed separation of the chromosomes, three-group metaphases, dispersal of relatively unchanged and supercoiled chromosomes, and "star" metaphases. The appearance of

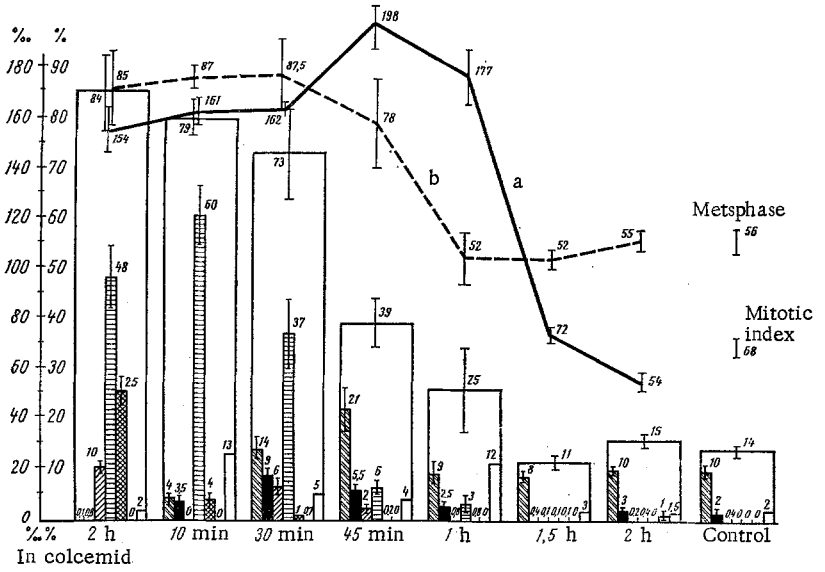

Fig. 2. Mitotic activity of fibroblasts of Chinese hamster of line 451 during reversal of the colcemid effect (after its action for 2 h in a dose of 0.03 μg/ml). Legend as in Fig. 1.

typical c-mitoses, it is interesting to note, was preceded by an increase in the number of pathological mitoses, including delayed separation of the chromosomes in metaphase and three-group metaphases.

A characteristic feature of "star" metaphase is that in this form of pathology of mitosis, unlike in the maternal star during normal mitosis, the chromosomes are concentrated in the center of the dividing cell (Fig. 3a). The kinetochores of the chromatids turned toward the center, facing the unseparated centrioles, are connected with them by several strands of the division spindle [3, 5]. Together with "star" metaphases, pseudoanaphases (or multiple "stars") could be observed. This type of c-mitosis is characterized by the formation of two or more stellate groups of metaphase chromosomes (Fig. 3b). The number of chromosomes in the groups may vary [2, 7].

Metaphases with delayed separation of the chromosomes and three-group metaphases disappeared quickly (by 30-45 min of exposure of the cells in colcemid solution). During the first hour of action of the alkaloid two types of c-mitoses were predominant: cells with dispersal of supercoiled chromosomes and "star" metaphases.

By the end of the first hour of exposure to colcemid the number of "star" metaphases fell slightly, and side by side with an increase in the number of cells with dispersal of supercoiled chromosomes there was an increase in the number of dividing cells in which the chromosomes were either clustered into a ball or formed a compact coil. The difference between the "ball" metaphase and the ordinary metaphase is that the chromosomes do not form an equatorial plate, although they likewise are not dispersed haphazardly (Fig. 3d). The chromosomes are spread all over the cell and form a loosely packed ball [4, 7]. The extreme form of pathology of mitosis is that in which all chromosomes were fused into one or several clumps in the center of the cell (Fig. 3e).

During the development of the colchicine mitosis there is thus a gradual succession of predominant forms of pathology of mitosis. Whereas in the early stages of action of colcemid delayed separation of the chromosomes in metakinesis and three-group metaphases appear initially, these are followed by the appearance of the early forms of c-mitosis ("star" metaphases) and, later, by dispersal of supercoiled chromosomes. After exposure to colcemid for 1 h the number of "ball" metaphases is increased and clumped metaphases appear. Presumably the succession of different forms of c-mitosis during development of the antimitotic effect is connected with variation in the degree of injury to the mitotic apparatus. Electron-microscopic studies have shown that differences in the degree of disorientation of the chromosomes correspond to differences in the degree of injury to the microtubules of the mitotic apparatus [16]. It has also been shown that if only the continuous microtubules are disorganized, only "star" metaphases arise,

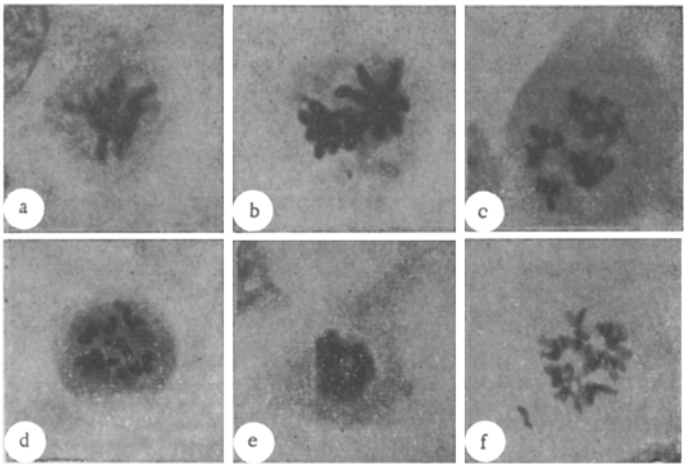

Fig. 3. Fibroblasts of Chinese hamster of line 451 treated with colcemid: a) "star" metaphase; b) pseudoanaphase; c) dispersal of supercoiled chromosomes; d) "ball" metaphase; e) clumped metaphase; f) dispersal of chromosomes. Carazzi's hematoxylin, 900×.

whereas if all microtubules are completely destroyed, dispersal of the chromosomes takes place [3]. Examination of the specimens always reveals a number of intermediate forms between the principal types of c-mitosis.

To study recovery of the normal course of mitosis, after exposure for 2 h to colcemid solution the cells were transferred to growth medium not containing the alkaloid, and the mitotic indices were studied by fixation at various times thereafter (Fig. 2).

Exposure to colcemid for 2 h caused a complete blocking of division at the metaphase stage ($P < 0.001$), and all the dividing cells consisted of c-mitoses: "star" metaphases, dispersal of supercoiled chromosomes, "ball" metaphases, and clumped metaphases.

From 20 to 30 min after the colcemid had been washed from the cells the mitotic index rose ($P < 0.001$) and at the same time there was a decrease in the number of metaphases ($P < 0.001$) and pathological mitoses ($P < 0.001$). The number of c-mitoses fell and metaphases with delayed separation of the chromosomes and three-group metaphases began to appear ($P < 0.001$).

The first ana-telophases began to appear 45 min after removal of the colcemid and their number increased gradually (up to 30% of the total of mitoses 60 min after removal of the colcemid).

The mitotic index reached its maximum 45-60 min after removal of the colcemid, after which it fell sharply to reach the control level 1.5-2 h after the cells had been kept in colcemid-free medium.

At 1.5-2 h of the recovery period all indices of mitotic activity had regained the control level. Consequently, restoration of the normal course of mitosis (like the development of c-mitoses) takes place through the appearance of a defect of mitosis connected only with partial injury to the division spindle.

After exposure to colcemid (from 2 min to 6 h) no significant change was observed in the number of multinuclear and polyploid cells, which according to the literature [11, 18] appear only after exposure to the alkaloids for 6-8 h.

The results of these experiments thus show that during development of the antimitotic effect of colcemid there is a regular succession of various phases of c-mitosis, probably resulting from different degrees of injury to the mitotic apparatus and chromosomes. After removal of the alkaloid the normal course of mitosis is restored.

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