EFFECT OF A SUBOPTIMAL TEMPERATURE ON THE MITOTIC REGIME

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Keeping Chinese hamster cells for 2 h at a suboptimal temperature (21°C) leads to a fall in the mitotic index and delay of division at the metaphase state. Cooling cause a sharp increase in the number of pathological mitoses, mainly as a result of injury to the mitotic apparatus: C mitoses and dispersion of the chromosomes in metaphase. After transfer of the cells to optimal temperature conditions the mitotic regime is completely restored after 1 h, but at this time the number of pathological mitoses is still appreciably greater than in the control.

KEY WORDS: *cell culture*; *mitotic regime*; *pathological mitoses*; *suboptimal temperature*.

Several investigations into the action of low temperatures on preparation of cells for division and on the course of mitosis [3, 4, 10, 13] and on survival of cells and the restoration of cell division after prolonged exposure to suboptimal temperatures [6, 14] have been published. The effect of keeping cells for a short time at suboptimal temperatures, however, has received little study. Yet this is an interesting problem in connection with the study of mechanism of mitosis, for we know that brief exposure to a suboptimal temperature induces reversible destruction of the microtubules of the mitotic spindle [5].

The object of this investigation was to identify the mitotic regime of a cell culture after exposure for 2 h to a suboptimal temperature.

EXPERIMENTAL METHOD

A transplantable line of Chinese hamster fibroblast-like cells (clone 237) was used. The cells were seeded in pencillin flasks with cover slips in a density of 2×10^5 cells/ml and were grown at 37°C on Eagle's medium with the addition of 10% bovine serum. After 24 h the culture were kept for 2 h in an incubator at 21°C and the cover slips with cells growing on them were then transferred to medium heated to 37°C. Material was fixed before exposure to the suboptimal temperature, after exposure for 2 h, and every 10 min after transfer of the cells back to optimal temperature conditions. The mitotic index (in promille), the ratio between the phases of mitosis, and the number of pathological mitoses, in %, were determined in preparations stained with Carazzi's hematoxylin [1]. No fewer than 5000 cells were counted at each time and the results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

As a result of exposure for 2 h to a suboptimal temperature the mitotic index fell from 53.6 to 36.4‰ ($P > 0.001$). The ratio between the phases of mitosis was sharply altered. Lowering the temperature caused delay of division at the metaphase stage, as manifested by the accumulation of metaphases and the total disappearance of anaphases and telophases (Fig. 1). The number of metaphases reached 84% after cooling, whereas normally metaphases account for about 45% of dividing cells ($P < 0.001$). The number of prophases was significantly reduced (from 26.5 to 15.4%, $P < 0.001$), probably on account of delay of the entry of the cells into mitosis when the temperature was lowered [12, 16]. Besides the change in the ratio between the phases of mitosis during cooling there was also a considerable increase in the number of

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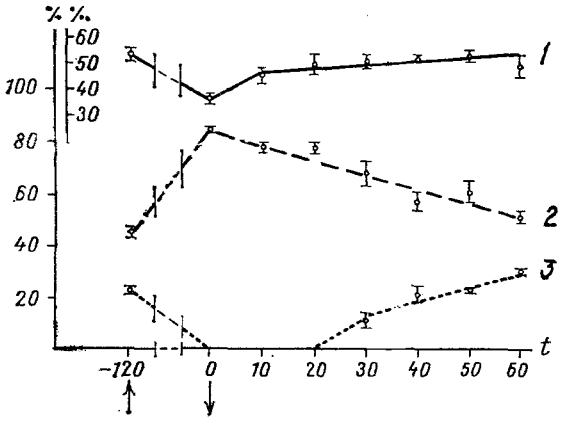

Fig. 1. Changes in mitotic regime of Chinese hamster cell culture after exposure to a suboptimal temperature for 2 h: 1) mitotic index (in °/₀₀); 2) number of metaphases; 3) number of anaphases and telophases (in %). Arrows indicate beginning and end of cooling. Abscissa, time, in min; ordinate, number of phases of mitosis (in %) and mitotic index (in °/₀₀).

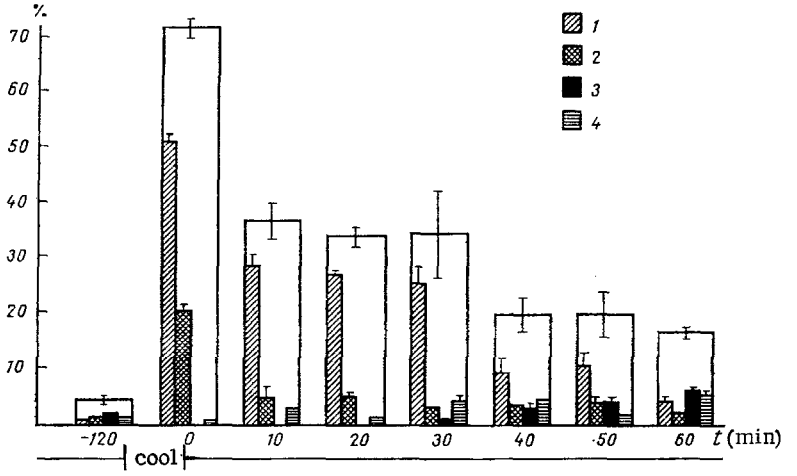

Fig. 2. Changes in number of pathological mitoses after exposure for 2 h to suboptimal temperature. Wide columns indicate total number of pathological mitoses; narrow columns: 1) C mitoses; 2) dispersion of chromosomes in metaphase; 3) bridges; 4) other types of pathology of mitosis. Abscissa, time (in min); ordinate, number of pathological mitoses (in %).

pathological mitosis (Fig. 2). For instance, under normal conditions the number of pathological mitosis after 24 h in culture usually does not exceed 4.5%, whereas after exposure for 2 h to a suboptimal temperature their number was increased almost 15-fold to 72% ($P < 0.001$). Pathological mitoses were represented mainly by forms connected with injury to the mitotic apparatus: various types of C mitoses and dispersion of the chromosomes in metaphase. The appearance of these forms of pathological mitosis can be explained on the grounds that lowering the temperature affects the process of polymerization of the microtubules, shifting the dynamic equilibrium between whole microtubules and the pool of subunits towards the latter [9]. Besides its effect on the formation of the mitotic spindle, the suboptimal temperature also probably acts on nucleoprotein metabolism of the cell, as indicated by the appearance of hyperspiralized or fused chromosomes, characteristic of C metaphases.

After transfer of the cells to optimal temperature conditions, the normal mitotic regime began to be restored. The mitotic index increased gradually to reach the control value after 40 min. The level of metaphases fell. The number of prophases increased rapidly and after

20 min was close to normal again. At that time the first anaphases and telophases began to appear. The normal course of mitosis was completely restored after 1 h. By that time not only the mitotic index, but also the ratio between the phases of mitosis had regained its initial values.

The total number of pathological mitoses fell gradually, but by the time of recovery of the mitotic regime the number of pathological mitoses was still appreciably higher than in the control. During restoration of the course of mitosis, while the number of C mitoses and dispersion of the chromosomes diminished, the number of bridges (pathological forms due to injury to the chromosomes) increased. The number of bridges 1 h after transfer of the cultures back to optimal conditions was 5.7% of all dividing cells, whereas before cooling their number did not exceed 2.0% ($P < 0.05$). During the recovery period the number of multipolar mitoses and the number of deletions of chromosomes in metakinesis and during divergence towards the poles increased (1.0% normally, 5.0% 1 h after the beginning of recovery; $P < 0.05$). The reason for the increase in the number of bridges during the period of normalization of the mitotic regime is not clear. As a rule bridges are the result of fragmentation of chromosomes, but in these experiments there was no increase in the frequency of fragmentation. Perhaps during exposure to suboptimal temperatures bridge formation took place in a way similar to that observed after treatment with ethidium bromide, i.e., by fusion of chromosomes followed by their breakage in anaphase [11]. In the present experiments fusion of the chromosomes also was observed after exposure for 2 h to a temperature of 21°C.

The results of this investigation agree with those obtained by other workers who observed a stathmokinetic effect of suboptimal temperatures [7] and the appearance of a broad spectrum of pathological mitoses (C mitoses, fragmentation, pulverization, fusion of chromosomes, etc.) in cultures of mammalian and avian cells in response to lowering of the temperature [6, 8, 15]. The changes in the mitotic regime observed in the present investigation after suboptimal cooling are very similar to those arising under the influence of colchicine (delay of division at the metaphase stage, an increase in the number of pathological mitoses mainly on account of C mitoses). However, the rapid recovery of the normal course of mitosis after exposure to a suboptimal temperature, by contrast with that observed after treatment with colchicine [2], suggests that the stathmokinetic effects of cooling and of colchicine are based on different mechanisms.

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