DURATION OF THE MITOTIC CYCLE OF CHINESE HAMSTER CELLS CULTURED AT 30-39°C

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The duration of the mitotic cycle (T) and of its stages at temperatures of 30, 33, 36, and 39°C was studied by an autoradiographic method in Chinese hamster cells of subline 237. The duration of the mitotic cycle was shortest at 39°C and increased as the cultivation temperature fell. Within the temperature range 33-39°C the increase in duration of the mitotic cycle and its stages was "proportional" to the temperature studied. The slope of the curve of duration of the mitotic cycle as a function of temperature increased sharply as the temperature changed from 33 to 30°C. The G_1 period was most sensitive and the G_2 period least to a change in the cultivation temperature.

KEY WORDS: *Cell culture; mitotic cycle; temperature dependence; periods of the cell cycle.*

Mammalian cells *in vitro* can pass through mitosis over a wide range of temperatures [2, 3, 5, 6, 8, 9]. However, few investigations have yet been undertaken to determine the effect of cultivation temperature on the various periods of interphase with the use of autoradiographic methods of analysis of labeled DNA precursors in the cell [8, 9, 11-13]. Information on the duration of the mitotic cycle at different temperatures would help to explain some aspects of the intracellular synthesis and the supply of energy for its reactions [10]. Such information would enable an experimenter to regulate accurately the "dosage" of the factors, conditions, etc. he is studying at the most sensitive period of life of the cell, by controlling its duration with the aid of temperature.

Data on the duration of the mitotic cycle of Chinese hamster cells in culture at temperatures ranging from 30 to 39°C are described in this paper.

EXPERIMENTAL METHOD

Chinese hamster fibroblasts of strain Blld-u-FAF 28, clone 237, were cultured on coverslips in flasks in the usual way in Eagle's medium with 10% bovine serum and antibiotics. Cultures seeded from one cell suspension in a concentration of 5×10^4 cells/ml were grown at 30, 33, 36, and 39°C. In the phase of logarithmic growth of the cultures they were pulse labeled in the usual way [1] for 20 min with thymidine-^{3}H in a concentration of 0.5 μCi/ml (specific activity 25 Ci/mmole). After washing to remove labeled thymidine the cultures were fixed at intervals of 2-3 h for 26-48 h in alcohol and acetic acid (3:1). Colchicine was added to the culture medium 2 h before fixation in a final concentration of 0.75 μg/ml. Autoradiographs were prepared with type M emulsion and stained with azure–eosin. At each test point 200 labeled metaphases were counted and the percentage of labeled mitoses calculated. The duration of the mitotic cycle (T) and of its individual periods (S, G_1, G_2) was determined graphically [11].

EXPERIMENTAL RESULTS

The dynamics of labeled mitoses after pulse labeling of cultures grown at 30-39°C is shown graphically in Fig. 1. The values obtained by analysis of these curves are given in

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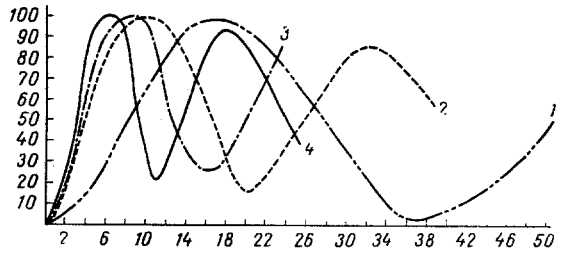

Fig. 1

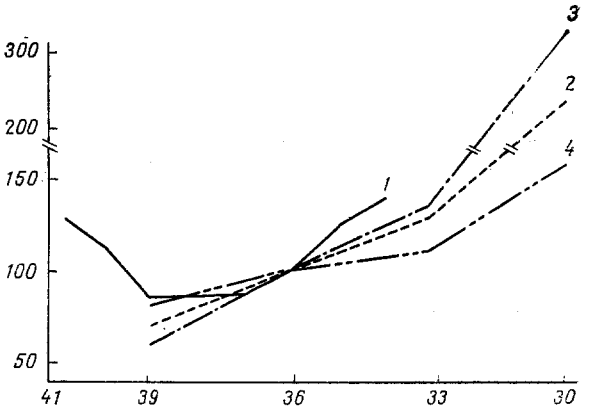

Fig. 2

Fig. 1. Changes in percentage of labeled mitoses in cultures of Chinese hamster cells (subline 237). Abscissa, time of cultivation after pulse labeling (in h); ordinate, percentage of labeled mitoses. 1) 30°C, 2) 33°C, 3) 36°C, 4) 39°C.

Fig. 2. Mean duration of mitotic cycle and its periods (in %) at 36°C. Abscissa, cultivation temperature (in °C); ordinate, duration of periods of mitotic cycle (in %). 1) T, 2) S, 3) G_1, 4) G_2.

TABLE 1. Duration of Mitotic Cycle and Its Periods for Chinese Hamster Cells at 30-39°C

Temperature, °C	Duration, h			
	T	G_1+M	S	G_2
39°	13,5	4,3	6,0	3,2
36°	19,5	7,0	9,0	3,5
33°	25,5	9,0	12,5	4,0
30°	51,0	23,5	21,0	6,5

Legend. T) Total mitotic cycle, G_1 + M, S, G_2) periods of mitotic cycle.

Table 1, from which it can be seen that the shortest duration of the mitotic cycle and of its individual periods occurred at 39°C. At a lower temperature the duration of all stages of the cycle was increased. The reaction of the early periods of the mitotic cycle to a change in temperature conditions of growth of the cells differed. The presynthetic period at 30°C was 3.5-5.5 times longer than at 39°C, the S period was 2.5-3.5 times longer, and the G_2 period only 2-2.3 times longer. The results of these experiments thus support the previous conclusion that the G_1 period is the most thermosensitive period [11-13].

Within the range of temperatures studied a fall or rise in temperature by the same number of degrees was not always accompanied by a proportional change in the duration of the periods of the mitotic cycle (Fig. 1). The graph shows that between 33 and 39°C changes in the values studied fell approximately on a straight line with a slope that changed sharply during the transition to 30°C. This reflects a sharp increase in the duration of the mitotic cycle, not comparable with that within the range 33-39°C, in the cell population formed at 30°C.

Similar "temperature-dependent anomalies" [5] have been described for several unicellular organisms [4, 5]. In work with suspension cultures of L 929 cells grown at 27-40°C, Lau [5, 6] showed the presence of two critical points at 30.2°C and above 37°C, at which the time taken for the cell population to double increased sharply. In the present experiments the lower point of the sharp increase in duration of the mitotic cycle was clearly apparent. The upper critical temperature for Chinese hamster cells presumably lies above 39°C. A similar picture also is observed on graphic analysis of the temperature dependence of the mitotic cycle of human amnion cells (Fig. 2), based on the results of Sisken et al. [12]. The differences from results obtained by Lau can perhaps be attributed to specific (species-specific?) differences between the cells.

There is as yet no generally accepted theory to account for differences in the response of cell populations to changes in ambient temperature conditions over a wide range of temperatures suitable for mitosis [6]. Without going into a detailed discussion of this problem, it may be simply pointed out that the results obtained for the duration of the mitotic cycle of Chinese hamster cells at 30-39°C in the present experiments show that the presynthetic period makes the main contribution to the sharp increase in the duration of interphase at the "critical" temperature.

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CHANGES IN MITOTIC ACTIVITY IN THE CORNEAL EPITHELIUM OF RATS OF DIFFERENT AGES AFTER NOCICEPTIVE STIMULATION

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The effect of nociceptive stimulation on mitotic activity in the corneal epithelium was investigated in 21-day-old rat fetuses and in rats aged 3, 4, 5, 7, 10, 15, 20, and 25 days. Mitotic activity was not significantly changed 45 min after nociceptive stimulation of the animals (amputation of one-third of the tail) in the cornea of the fetuses and day-old rats. Between the 3rd and 10th days of postnatal development reactive inhibition of mitosis in response to nociceptive stimulation was gradually formed. After 10 days this response was intensified and reached a maximum by the 25th day. Reactive inhibition of mitotic activity is connected with delayed entry of the cells into mitosis.

KEY WORDS: *Corneal epithelium; nociceptive stimulation; mitotic index.*

Numerous investigations have shown that in response to the action of various stressors (electric shock, nociceptive stimulation, and so on) reactive inhibition of mitosis is observed in the ectodermal tissues of mammals [4-11]. Adrenalin secreted by the adrenals during excitation of animals has been shown to play in important role in the mechanism of this response. Meanwhile, other investigations have shown the absence of reactive inhibition of

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