TEMPERATURE LIMITS OF MITOSIS IN MAMMALIAN CELL CULTURES

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UDC 612.014.3:612.6].014.43

To determine the temperature limits of mitosis 11 strains of mammalian cells of different origin were grown in culture between temperatures of 25 and 41°C. Different strains of mammalian cells were shown to respond differently to a rise and fall of the cultivation temperature. The upper temperature limit lies not more than 5° above the optimum and a decrease of temperature of more than 10°C does not stop cell division. In most cell populations there are three types of cells. Most cells can divide within a certain range of temperatures, a smaller group of cells can start mitosis but not complete it, the process being stopped in metaphase, and a very small number of cells can complete one division at below the threshold temperature. The temperature limits of mitosis are unconnected with the species or tissue origin of the cells.

KEY WORDS: cell and tissue cultures; mitosis.

A temperature of 37 ± 1.0°C is regarded as optimal for cultures of cells from warm-blooded animals. The temperature limits within which normal karyokinesis of mammalian cells is possible have not yet been finally established. The upper temperature limit lies between 42 and 43°C [1, 6, 7, 11], but the boundary of the suboptimal temperatures at which mitosis is possible varies within wide limits according to data obtained by different workers [2-5, 9, 10].

This paper gives the results of experiments to study the temperature threshold of cell division.

EXPERIMENTAL METHOD

Experiments were carried out on 11 cell strains of different origin (Table 1). Nutrient medium (Eagle's or No. 199 with 10% bovine serum) and the cell concentration for seeding ($0.3 \cdot 10^5$-$2.5 \cdot 10^5$ cells/ml) were chosen depending on the type of cell and the temperature to be studied; the cells were grown without any change of medium. The criteria of cell multiplication were: the dynamics of the cell population density, the mitotic index (MI), and the number of metaphases accumulating 2, 4, 8, and 12 h after addition of colchicine in a concentration of 0.5-1.5 µg/ml to the nutrient medium (MI_{col}). The range of temperatures studied was 25-36°C, but some strains of cells were grown at 39-41°C.

EXPERIMENTAL RESULTS

The experiments showed that the lower limit of temperature at which normal mitotic division can take place differs for different cells. Values of MI at the height of mitotic activity of cultures growing at 36-26°C are given in Table 1. MI clearly increased with a decrease in the cultivation temperature; only at 26°C was there a sharp decrease in MI. On the basis of these results it might be supposed that the cells of all strains could complete mitosis at 28°C. However, the density of the cell population increased only at temperatures

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 81-84, December, 1975. Original article submitted February 28, 1975.

TABLE 1. MI (in %) in Cell Cultures at Different Temperatures

Strain of cells*	Temperature (in °C)						Temperature at which colchicine acts (in °C)
	36	34	32	30	28	26	
L	16,0±1,22	16,7±1,21	19,9±1,48	23,0±1,30	28,6±1,92	5,2±0,71	27
451	24,7±2,58	28,0±2,20	—	36,6±2,17	40,0±4,76	33,6±3,6	26
237	27,2±1,48	29,8±1,38	—	31,9±1,55	34,3±2,07	14,7±1,35	27
VNK	22,5±1,67	26,0±3,00	29,6±1,85	34,4±3,31	41,3±4,89	10,5±1,30	27
A-1	28,1±3,09	—	—	—	43,7±4,89	—	—
SPEV	24,0±1,65	—	30,0±2,35	35,5±3,75	32,0±2,53	—	29
Hep-2	11,0±1,09	18,0±1,36	21,5±2,32	13,0±2,36	2,7±0,45	—	30
MK-2	14,0±1,58	—	16,0±1,66	18,0±1,09	20,0±1,94	12,0±1,08	30
RH	23,1±2,02	51,8±5,71	78,1±6,06	82,0±7,42	81,0±4,90	26,0±1,90	33
Wish	18,3±1,28	32,6±2,32	52,4±4,57	80,2±8,06	65,3±5,22	16,8±1,55	33
F1	26,4±1,22	—	34,2±2,97	52,8±5,33	45,4±2,47	—	—

*L) Mouse fibroblasts; 451 and 237) substrains of Chinese hamster cells; VNK) Syrian hamster cells; A-1, F1, and Wish) human amnion cells; RH) human kidney cell; Hep-2) human laryngeal carcinoma cells; MK-2) monkey kidney cells; SPEV) pig embryonic kidney cells.

TABLE 2. Changes in Value of MI (in °/oo) During Cultivation of Cells at 26 and 25°C

Strain of cells	Temperature and times of cultivation							
	26°				25°			
	4 h	1 day	2 days	3 days	4 h	1 day	2 days	3 days
L	2,8±0,31	4,6±0,74	5,2±0,71	3,2±1,04	3,1±0,65	5,0±0,96	4,5±0,44	2,9±0,95
451	8,0±1,02	13,5±1,26	20,3±2,68	33,6±3,60	7,8±0,70	12,3±1,45	16,0±1,22	19,6±1,28
RH	3,8±0,56	10,0±1,05	16,0±1,81	26,0±2,31	4,5±0,89	8,0±1,14	16,0±2,56	18,6±2,00
Wish	3,5±0,89	8,0±1,14	16,8±2,57	—	—	—	—	—

to the left of the line dividing the table. At temperatures to the right of the line, despite the high MI, the population cell density not only did not increase but, on the contrary, it started to fall sharply after 48 h of cultivation. Mitotic figures in these cultures consisted chiefly of metaphases, with only very occasional prophases; the percentage of anaphases and telophases was very small (3-5% compared with 2% at 36°C). Suboptimal temperatures produced a mitostatic effect in the cultures: Some cells started to undergo mitosis but did not complete it, stopping in metaphase; addition of colchicine did not therefore lead to any marked increase in MI. In the last column of Table 1 temperatures at which MI increased proportionally to the duration of action of colchicine are shown. These temperatures can be regarded as threshold temperatures for mitosis. They are 1-2°C lower than temperatures at which the cell population density increased. At the threshold temperatures the number of cells was unchanged in the course of 3-5 days and evidently equilibrium was established in the population between growth and death of the cells, such as has been described in a culture of HeLa cells at 31°C [9, 10].

In most cell strains with a temperature threshold of 28°C, besides cells with mitosis blocked in metaphase, there was a small group of cells capable of completing at least one subdivision at subthreshold temperatures. This is shown by the presence of up to 5% of anaphases and telophases and by an increase in MI_{col} (up to 20%).

Changes in MI for certain cell strains at 25 and 26°C are shown in Table 2. Clearly in the culture of L cells the value of MI was unchanged, but in the others MI increased during cultivation. However, anaphases and telophases (up to 5%) were found only in cultures of Chinese hamster cells. Possibly some cells of these cultures could also divide at 25°C, a certain number of RH and Wish cells could begin mitosis but not finish it, whereas there were practically no such cells in the L strain.

Cells of different strains differed in their response to a rise of temperature. Experiments in which cells of the substrains 451 and 237 were grown at 39 and 41°C showed that in both cases in the cultures of Chinese hamster cells there was an increase in the cell popu-

lation density. In the culture of L cells at 41°C the number of cells was unchanged during the first 2 days. In that case, just as at the lower temperature threshold, temporary equilibrium was established between growth and death of the cells.

These experiments showed that populations of most mammalian cell strains react differently to a rise or fall of the cultivation temperature. Whereas the upper limit of temperature for mitosis is not more than 5°C above the optimum, a fall of temperature by more than 10°C does not stop karyokinesis of several strains of cells. Many cells of the population can divide within a certain range of temperatures, a smaller group of cells can start mitosis at the subthreshold temperature but not complete it, being held up in metaphase, and, finally, a very few cells can complete one (?) mitosis at a lower temperature. Cells capable of dividing under these conditions are absent in cultures of L and Hep-2 cells.

The temperature limits of mitosis are independent of the species or tissue origin of the cells but are evidently determined by the life history of each cell strain. The presence of cells responding identically to suboptimal temperatures in the populations is evidence of the temperature instability of these cell strains and that the temperature threshold of karyokinesis may vary in the course of their subsequent cultivation *in vitro*.

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