RESTORATION OF CELL DIVISION IN TISSUE CULTURES AFTER COOLING

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Recovery of mitotic activity and increase in cell population were studied in a culture of L cells after storage at suboptimal (20-30 ± 0.5°) and low (4 ± 1°) temperatures for 3-30 days. Mitotic activity recovered more rapidly after storage at 20-24° than at 4°. A temperature of 30° does not stop mitosis, and a gradual increase in the number of cells is observed, so that they can be cultivated without change of medium for 20 days or longer. The number of pathological mitoses is increased in cooled cultures; their percentage depending on the duration of storage. Suboptimal temperatures cause adhesion of chromosomes with the appearance of K metaphases, and after exposure to low temperatures the percentage of multipolar mitoses is increased.

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Storage of grown tissue cultures or cell suspensions at room temperature or in a refrigerator allows many cells to be preserved for 2-4 weeks [5, 9]. Recovery of cell populations when stored under different temperature conditions takes place differently [1]. In the present investigation mitotic activity was studied in a culture of cells after cooling which varied in degree and duration.

EXPERIMENTAL METHOD

L cells were grown at 36° in medium No. 199. On the 2nd-3rd day of cultivation they were transferred to a refrigerator at 4 ± 1°, or to suboptimal temperatures (20, 22, 24, 26, and 30 ± 0.5°). On the 3rd, 5th, 7th, 9th, 12th, and 14th days of storage the flasks containing the cell culture were incubated at 36° and samples taken every 2 h until 40 h inclusive, and again after 3 and 5 days were fixed in Bouin's fluid and stained. Mitoses in 5000 cells were counted in the stained preparations and the mitotic index (MI) expressed in promille and the number of aberrant mitoses in percent.

To discover whether L cells grown in 100-ml flasks could be stored for long periods, they were kept at the above-mentioned temperatures for 20 and 30 days. Before the cultures were stored away, the yield of cells was determined in 4 or 5 flasks. At the end of the storage period the number of surviving cells was counted, and the increase in the cell population was determined on the 3rd, 5th, 7th, and 9th days of cultivation.

EXPERIMENTAL RESULTS

Propagation of cells stored at 20-24° takes place relatively faster than in cultures stored at 4°. The lag phase of the latter lasts 5 days or more, and the proliferation index (ratio between number of grown cells and number seeded) on the 9th day of cultivation was 2.5:4.5. After exposure to suboptimal temperatures (20-24°) the lag phase ended on the 3rd day, and the proliferation index varied from 1.5 to 3.5 on the 5th day, reaching 6-8 on the 9th day. Prolonged storage of the cultures at 4° caused rounding of the cells and destruction of the monolayer; after 12 days practically all the cells were suspended in the medium. Evidently not all "living" cells after storage at 4° were capable of proliferation. This is attributable to irreversible depression of adhesiveness of the cells by prolonged exposure to cold [2, 8]. After storage of the cultures for 72 h at 4°, restoration of cell division during subsequent cultivation at 36° took place slowly. The mitotic index reached the control level after 26-30 h (Fig. 1). Storage for longer periods caused deeper inhibition of mitotic activity. Its recovery in cultures stored at 20-24° took place rapidly

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TABLE 1. Mitotic Activity (in $^0/_{00}$) During First Hours of Cultivation of Cooled Cells and Number of Pathological Forms of Mitosis (M±m)

Temperature (in deg.)	Duration of exposure (in days)	Mitotic activity after					Percentage of aberrant forms of mitosis[1]			
		2 h	4 h	6 h	8 h	10 h	total	adhesions	K-metaphases	multipolar
20—24	3	6,2±1,87	18,1±2,88	42,5±3,78	25,0±2,75	15,?±1,45	6,6±0,8	0,2	0	1,3
	5	7,6±1,65	12,5±2,30	55,1±3,71	34,4±1,52	18,6±2,28	12,0±0,95	1,2	0,7	1,7
	7	3,8±0,63	16,3±2,29	24,5±2,61	50,3±3,28	33,3±3,63	28,5±4,5	4,2	5,6	2,4
	9	1,3±0,63	11,8±3,55	24,0±3,72	45,6±3,72	30,8±1,65	37,0	14,5	6,5	2,0
	12	0	6,0±1,24	7,9±1,65	15,2±3,05	30,2±2,05	70,0	37,0	9,0	1,2
	14	0	0	5,9±0,79	12,6±1,45	32,5±3,44	81,0	53,0	14,0	1,0
26	3	11,6±1,43	13,4±1,05	13,2±2,38	12,0±1,84	10,0±1,63	10,5±2,21	1,0	0	2,0
30	3	15,3±3,17	16,1±2,12	15,4±2,52	11,9±1,94	11,3±2,49	7,3±1,59	0	0	1,5
4	3	3,9±1,57	5,6±1,73	13,6±1,62	9,0±1,96	8,2±1,90	11,9±2,82	0	0	5,1
4	5	0	3,6±1,64	11,8±2,24	11,0±1,98	8,7±1,97	16,8±2,88	0	0	6,0

[1]Percentage determined at height of mitotic activity.

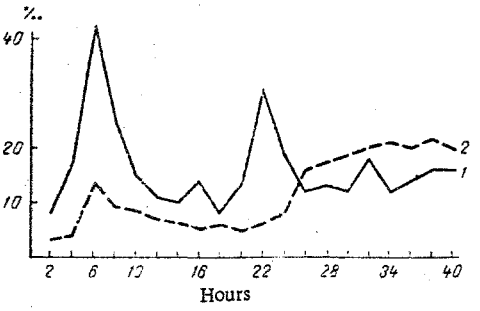

Fig. 1. Mitotic index in culture of L cells after cooling for 3 days. Abscissa, cultivation time (h); ordinate, mitotic index (in $^0/_{00}$). 1) 22 ± 0.5°; 2) 4 ± 1°.

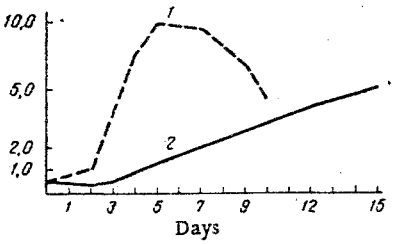

Fig. 2. Changes in number of L cells at 36° (1) and 30° (2). Abscissa, duration of cultivation (in days); ordinate, number of cells per ml, multiplied by 10^5.

with a well defined periodicity. The first peak of mitoses appeared after 6 h, and the second after 22 h of cultivation (Fig. 1).

The mitotic index reached its highest value at these times after storage of cultures for 5 days. Longer exposure to this temperature displaced the waves of mitosis in time, so that after storage for 7-9 days the first peak of mitoses was found after 8 h, and after storage for 12-14 days it was later still (Table 1). Partial synchronization of mitoses which we observed was presumably due to the fact that at 20-24° synthesis of DNA continued in some cells [4]. By the 5th day of storage, the number of cells which had finished synthesizing DNA in the culture reached its maximum. Under these conditions the subsequent course of the mitotic cycle was blocked [6]. Transfer of the cells to cultivation at 36° removed the block; the cells passed into the G_2 phase and commenced mitosis. The second wave of mitoses was the result of the next division of these cells. The time between peaks of mitoses corresponds to the duration of the mitotic cycle, in this case 16 h, a value close to that determined for L cells by means of thymidine-H^3 [3]. The possibility that DNA synthesis may take place in nuclei of cells at 4°, suggested by certain workers [7], is slight judging from the resumption of mitotic activity.

The number of pathological mitoses was greatly increased in the cultures after cooling. Under optimal conditions the percentage of pathological mitoses in L cells is fairly constant: on the 2nd-4th day of growth 4.4 ± 0.75%, after aging of the cultures 6-8%. The number of aberrant mitoses in restored cultures increased with an increase in the period of stay of the cells at suboptimal temperatures (Table 1). Many K-metaphases, adhesions, and "pulverizations" of chromosomes, i.e., pathological forms not found in the control, appeared under these circumstances. After exposure to low temperatures, the number of multipolar mitoses increased.

Cultivation of cells under optimal conditions after storage at 26° and 30° was not accompanied by synchronization of mitoses. A temperature of 30° did not arrest mitosis [7]. Division of L cells can be maintained under these circumstances for 20 days or more without change of medium (Fig. 2). At 26°, no increase in the cell population took place.

The results show that the degree and duration of cooling of cell cultures determine the rate of recovery of subsequent mitotic activity, and also determine the changes on which depends the character of the regenerated cell population.

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