infiltration is reduced (by the 8th day), and early and more intensive proliferation of fibroblasts is observed. In the early stages (4th-8th day) the most favorable effect on the course of reparative regeneration is given by laser radiation with a power density of 4 mW/cm^2 and an exposure of 2 min.

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STATHMOKINETIC EFFECT OF CRYOPROTECTORS

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KEY WORDS: cryoprotectors; stathmokinetic effect; c-mitoses; cell cultures.

Investigations have shown that the action of certain factors, notably hypothermia, hydrostatic pressure, hyperconcentrated salt solutions, and antitubulins, lead to the appearance of a stathmokinetic reaction in cell systems, which is a reversible process [1, 2, 5, 6]. Exposure to the above-mentioned factors leads to a basically similar morphological picture, expressed as delay (blocking) of mitosis at the metaphase stage, and the appearance of so-called c-mitoses, characterized by a haphazard arrangement of supercoiled chromosomes in the cells.

In the course of our work we also have observed a metaphase block and the appearance of many c-mitoses in short-term cultures of human peripheral blood and transplantable monolayer cultures of Chinese hamster fibroblast-like cells after incubation with cryoprotectors — substances used during low-temperature conservation of various cell suspensions. However, no data could be found in the accessible literature on the stathmokinetic action of cryoprotectors, notably glycerol and polyethylene oxides (PEO-400).

The aim of this investigation was to study the stathmokinetic action of glycerol and PEO-400 during contact with cell cultures.

EXPERIMENTAL METHOD

The test material was a short-term culture of human peripheral blood leukocytes stimulated by phytohemagglutinin (PHA) and a monolayer transplantable culture of Chinese hamster

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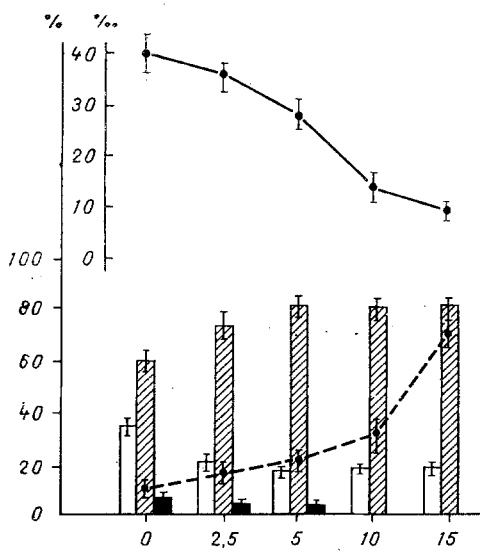

Fig. 1

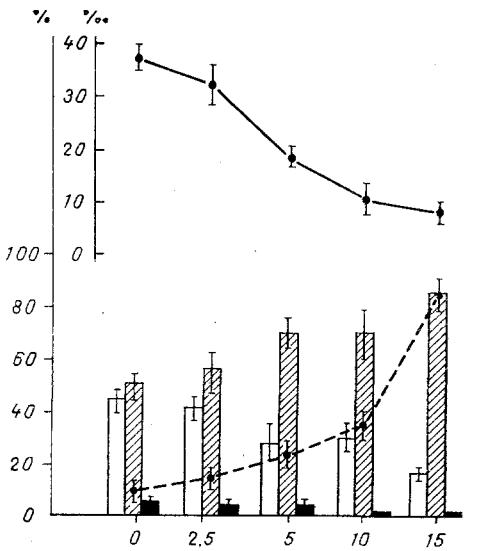

Fig. 2

Fig. 1. Changes in mitotic activity of short-term human peripheral blood leukocyte culture after incubation with glycerol. Abscissa, glycerol concentration (in %). Continuous line, MI (in °/oo). Unshaded columns, prophases, obliquely shaded, metaphases; black, ana-telophases (in % of corresponding MI). Broken line, number of c-mitoses (in % of corresponding MI).

Fig. 2. Changes in mitotic activity of short-term human peripheral blood leukocyte culture after incubation with PEO-400. Legend as to Fig. 1.

fibroblast-like cells (clone 237). Glycerol and PEO-400 were used as cryoprotectors. The cryoprotectors were added to the culture medium in 5, 10, and 15% final concentrations at the beginning of cell culture. Samples of cell cultures grown in medium without the addition of cryoprotectors were used as the control. The blood cells were cultured for 69 h at 37°C by the technique described previously [3]. Chinese hamster cells in culture were incubated with cryoprotectors in 5 and 10% concentrations for 4 h at 37°C in penicillen flasks on coverslips with a density of 2×10^5 cells/ml in Eagle's medium with 10% bovine serum. After incubation for 4 h the coverslips with cells settling on them were carefully washed in warm Hanks' solution; some of them were then fixed in a mixture of alcohol and acetic acid (3:1) and the rest were transferred to fresh nutrient medium, in which culture continued at the same temperature. This material was fixed after 2 and 24 h of growth after washing. Control cultures were subjected to the same treatment. The mitotic index (MI) in promille, the ratio between the phases of mitosis, and the number of c-mitoses as a percentage of the corresponding MI, were determined in films of blood cell cultures stained by the Romanovsky—Giemsa method and Chinese hamster cells stained with Carazzi's hematoxylin. All the numerical results were subjected to statistical analysis by the Student—Fisher method.

EXPERIMENTAL RESULTS

Addition of glycerol and PEO-400 to the culture medium of blood cells significantly reduced their MI (Figs. 1 and 2). Meanwhile, with an increase in concentration of cryoprotectors in the nutrient medium of these cultures to 15%, changes were observed in the relative percentages of the individual phases of mitosis: The number of prophases and ana-telophases was reduced with total absence of the latter in some cases. Analysis of the distribution of the individual phases of mitosis revealed a significant increase in the relative number of metaphases compared with the control. Meanwhile a considerable increase in the number of c-metaphases and anomalies of division, connected with injury to the mitotic apparatus, was observed (especially in 10-15% concentrations).

After 4-h incubation of Chinese hamster fibroblast-like cells with glycerol and PEO-400 a tendency also was observed for MI to fall compared with the control (Table 1). MI was particularly low (almost down to half or one-third of the control level) 2 h after rinsing of the cells. After 24 h of growth in fresh medium MI of cultures treated with 5% glycerol and 10% PEO-400 remained significantly below the control level. Analysis of the distribution of individual phases of mitosis showed that after incubation of the Chinese hamster cells for

TABLE 1. Mitotic Activity of Chinese Hamster Cells after Incubation with Cryoprotectors (M ± m, n = 5)

Treatment	Concentrations of cryoprotectors, %	Time after treatment, h	MI, ‰	Proportions of phases of mitosis, %			No. of c-mitoses, %
				prophase	metaphase	ana-telophase	
Control		0	21,8±2,1	20,2±3,0	53,0±3,5	26,8±1,8	3,5±0,6
Glycerol	5		18,5±2,1	4,8±2,9	82,2±6,2*	13,0±1,9	20,6±4,5***
PEO-400	5		17,0±2,6	8,3±2,4	76,1±3,3*	15,6±1,7	8,9±2,9
Glycerol	10		19,0±1,5	12,6±2,1	85,6±1,9***	1,8±0,3	18,3±2,5***
PEO-400	10		13,0±1,0*	15,0±1,8	74,3±4,2*	10,7±1,4	6,8±1,3
Control		2	22,8±2,9	21,3±3,5	47,8±3,4	30,9±3,5	1,6±1,3
Glycerol	5		14,8±3,9*	40,7±3,4	54,0±5,7	5,3±2,1	15,8±2,1**
PEO-400	5		10,8±2,1**	24,6±4,2	38,2±4,6	37,2±1,5	6,8±1,7
Glycerol	10		6,4±0,8**	12,5±1,3	59,0±3,0*	28,5±1,9	14,1±1,3**
PEO-400	10		8,7±2,0**	36,8±3,4	34,4±3,1	28,8±3,4	22,3±3,8***
Control		24	41,6±2,9	27,3±4,4	56,5±4,2	16,2±1,1	1,7±1,4
Glycerol	5		35,8±1,1	26,2±2,2	49,9±3,0	23,9±3,7	2,5±0,8
PEO-400	5		34,4±3,0	33,3±1,6	47,1±3,8	19,6±4,9	4,8±1,6
Glycerol	10		34,2±3,4*	19,8±3,7	53,8±3,7	26,4±1,2	8,6±1,6**
PEO-400	10		30,5±2,5*	18,1±1,7	60,1±3,0	21,8±1,6	15,5±3,4***

Legend. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

4 h with the cryoprotectors the number of prophases and ana-telophases decreased with an increase in their concentration whereas the number of metaphases increased significantly compared with the control. At later times of growth (2 and 24 h after rinsing), because of a new wave of dividing cells, the number of prophases increased whereas the number of metaphases and ana-telophases mainly reached the control values. The predominant form of pathology of division, just as in blood cultures, was the colchicine-like mitosis (c-mitosis), the relative percentage of which depended both on the concentrations and on the types of substance.

There is evidence that the stathmokinetic response of cells may arise by different mechanisms: injury and disorganization of microtubules, disturbance of the thiol mechanism of mitosis, disturbance of synthesis of proteins of the achromatin spindle [1, 2]. However, this list probably does not exhaust the mechanism of the stathmokinetic effect.

In an attempt to explain the possible mechanisms of inhibition of mitosis at the metaphase stage and the appearance of c-metaphases, we set out both from the dehydrating property of glycerol and PEO-400 and also from the ability of these substances to penetrate to some degree inside cells, and thereby to affect the formation of the mitotic apparatus [4]. The possibility likewise cannot be ruled out that glycerol and PEO-400 act indirectly on the nucleotide metabolism of cells, as is shown by the appearance of supercoiled and fused chromosomes, characteristic of c-metaphases.

After rinsing to remove the cryoprotectors, MI of the cell cultures and the ratio between the phases of mitosis were restored to the control values, evidence of normalization of the division spindle and of the ability of the cells to escape from metaphase block and to effect the final stages of mitosis (ana-telophase). However, the number of c-mitoses remained significantly higher than in the control in cultures of Chinese hamster cells, especially in those treated with cryoprotectors in a 10% concentration, evidence of the need to lengthen the culture time of the test cells after washing off the cryoprotectors.

Glycerol and PEO-400, in concentrations widely used to freeze biological objects, during long-term contact with cells in culture, thus exert a stathmokinetic action, which is reversible in character and is linked with their common dehydrating properties, although the degree of its manifestation depends on their penetrating power.

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CIRCADIAN RHYTHM OF MITOSIS IN THE CORNEAL EPITHELIUM OF *Microtus arvalis* PALLAS

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KEY WORDS: mitosis; mitotic index; corneal epithelium; vole.

Many factors and mechanisms participate in the regulation of mitotic activity (MA) of cells [4-6]. Cell multiplication processes, it must be assumed, are under the control of a combination of various factors, both external (environmental) and internal (in the body itself). In the study of mitosis great attention must be paid to ecologic factors and to the type of behavior of the animals themselves, which determine the character of manifestation of various processes at the tissue and cell level, i.e., to the motor activity of the animal itself [2].

Despite closer attention to the study of MA, it has not yet been adequately studied in certain organs of wild animals [3, 7].

The aim of this investigation was accordingly to analyze the dynamics of the circadian rhythm of mitosis in the corneal epithelium of adult male and female common voles (*Microtus arvalis* Pallas) during the summer in relation to the circadian rhythm of their motor activity.

EXPERIMENTAL METHOD

Voles caught in the Trakai District of Lithuania in July, 1981, were used. The cornea was chosen as test material because it is the easiest object to analyze. Meanwhile processes taking place in the cornea reflect many aspects of energy metabolism in the organism as a whole [8].

MA in the corneal epithelium was determined in adult animals. Voles weighing 20 g or more were considered to be adult. The mean weight of the animals was 30.0 ± 0.8 g (n = 64) for males and 31.7 ± 1.32 g (n = 91) for females. The animals were decapitated immediately after being caught and thereafter every 3 h for 24 h: at 3, 6, and 9 a.m., 12 noon, 3, 6, and 9 p.m., and midnight, and additionally at 1, 4, and 8 p.m. Total preparations of the corneal epithelium were obtained and the mitotic index (MI, in promille) was calculated by the usual methods [1].

EXPERIMENTAL RESULTS

In the corneal epithelium of the common vole MA is observed by both day and night and it has a tendency to exhibit a polyphasic rhythm (Tables 1 and 2). Altogether 18,786 mitoses were found in total preparations, of which 13,272 (70.6%) were in the period from 9 a.m. to 9 p.m. inclusive. In males, of the 6652 mitoses observed 4229 (63.6%) occurred during daylight, compared with 9043 (74.5%) of 12,134 mitoses recorded in females at that time. The mean MI at night for males was 5.67 ± 1.10‰ and during the day 3.65 ± 1.04‰, and for females 5.62 ± 1.43 and 4.75 ± 1.10‰ respectively. In the interval between darkness (3 and 6 a.m., and midnight) and daylight (9 a.m., noon, 1, 3, 4, 6, 8, and 9 p.m.) differences were not statistically significant, evidence that the level of MI is the same at these times.

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