stances for carcinogenicity by the use of organ cultures of embryonic target tissues [11]. The use of this criterion is evidently promising also for the detection and quantitative estimation of species, linear, and organ predisposition to the development of tumors.

LITERATURE CITED

1. L. A. Andrianov, G. A. Belitskii, Yu. M. Vasil'ev, et al., The Action of Carcinogenic Hydrocarbons on Cells [in Russian], Moscow (1971), pp. 93-98.
2. T. S. Kolesnichenko, Vopr. Onkol., No. 12, 39 (1966).
3. T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 5, 71 (1973).
4. T. S. Kolesnichenko, Vopr. Onkol., No. 8, 72 (1974).
5. T. S. Kolesnichenko, Vopr. Onkol., No. 5, 68 (1976).
6. T. S. Kolesnichenko and N. B. Gusina, Byull. Éksp. Biol. Med., No. 10, 426 (1978).
7. T. S. Kolesnichenko and N.V. Popova, Byull. Éksp. Biol. Med., No. 9, 1116 (1976).
8. T. S. Kolesnichenko, N. V. Popova, and L. M. Shabad, Byull. Éksp. Biol. Med., No. 12, 716 (1979).
9. N. V. Popova, Byull. Éksp. Biol. Med., No. 6, 732 (1977).
10. L. M. Shabad, T. S. Kolesnichenko, and Yu. D. Sorokina, Transplacental Carcinogenesis and Organ Cultures [in Russian], Moscow (1975).
11. T. S. Kolesnichenko and L. M. Shabad, Neoplasma, 6, 369 (1979).
12. P. Nettesheim, A. Marchok, and M. Terzaghi, in: Polycyclic Hydrocarbons and Cancer, (H. V. Gelboin et al., eds.), Vol. 2, New York (1978), pp. 307-329.
13. J. M. Quarles, M. W. Sega, C. K. Schenley, et al., Nat. Cancer Inst. Monogr., 51, 257 (1979).

EFFECT OF SUBLETHAL HYPERTHERMIA ON PROLIFERATION OF THE CORNEAL EPITHELIUM IN ALBINO RATS

M. I. Radivoz and S. S. Timoshin

UDC 612.841.1.014.2:612.6]-06:612.591

KEY WORDS: hyperthermia; proliferation of cells; homoiothermic animals.

Processes of cell proliferation in eukaryotes in vitro take place over a wide range of above-zero temperatures [7, 11]. Data on the effect of high temperatures on cell division in vivo in homoiothermic animals are few and inconsistent in character [10, 12].

Nevertheless the study of this problem is of considerable practical importance in connection with the use of hyperthermia in the treatment of diseases accompanied by disturbances of cell division [1], and this was the motivation behind the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-200 g. The animals, which were first adapted to the experimental conditions, were heated in a hot chamber at 42°C and with a relative air humidity of 60-65%. Exposure to a high temperature lasted 1.5 h until the rectal temperature was 41-41.5°C. The normal body temperature of the rats was restored 30-45 min after the end of hyperthermia. Considering the existence of a circadian rhythm of proliferation in the corneal epithelium in response to stressor stimulation [5, 8, 9], the animals were heated in the morning at 6-8 a.m., at midday between 11 a.m. and 1 p.m., and in the evening between 5 and 7 p.m. Mitotic activity was studied 2, 6, and 12 h after the end of exposure to heat. The number of animals in the experiment was 280. To assess the reproducibility of the results, all series of experiments were repeated twice. Total preparations were obtained, the mitotic index (MI) and level of patho-

Central Research Laboratory, Khabarovsk Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 8, pp. 222-224, August, 1980. Original article submitted January 4, 1980.

0007-4888/80/9008-1133$07.50

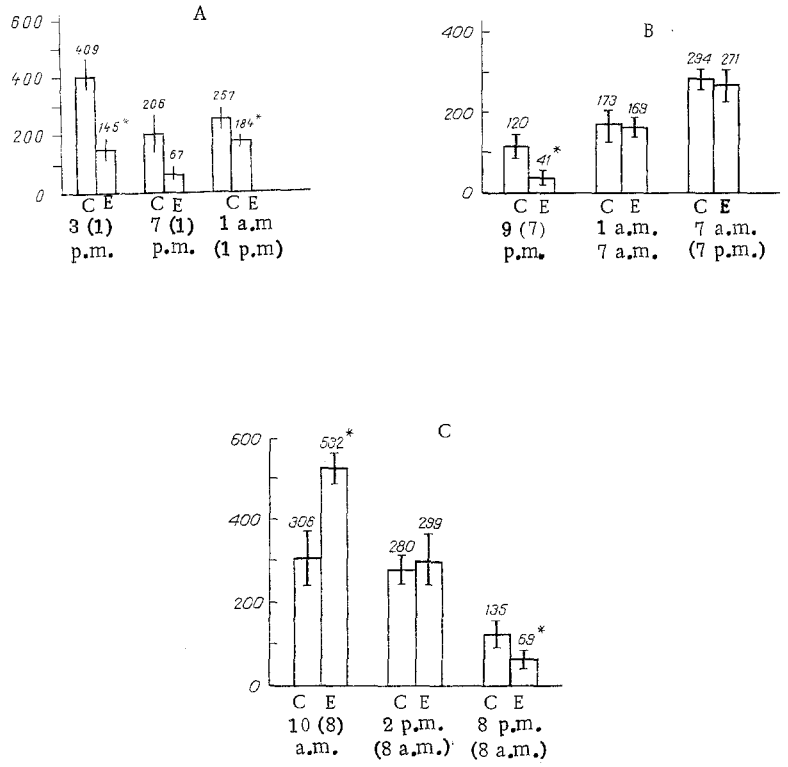

Fig. 1. Effect of sublethal hyperthermia at midday between 11 a.m. and 1 p.m. (A), in the evening between 5 and 7 p.m. (B), and in the morning between 6 and 8 a.m. (C) on mitotic activity of the corneal epithelium in albino rats. C) Control; E) experiment. Abscissa, time of end of hyperthermia (in parentheses) and time of sacrifice of animal; ordinate, number of mitoses in 100 fields of vision. *) $P<0.05$.

logical mitoses (PM) were estimated, autoradiographs prepared, and the index of labeled nuclei (ILN) and intensity of DNA turnover were determined by the method described previously [6]. The numerical results were subjected to statistical analysis by the Fisher–Student method and by the method of confidence intervals.

EXPERIMENTAL RESULTS

When rats were exposed to hyperthermia at 11 a.m.-1 p.m. a decrease in the number of dividing cells was observed 2, 6, and 12 h after the end of exposure to heat (Fig. 1A). MI of the experimental animals was 2.8, 3.0, and 1.3 times less respectively at these times than in the control. In rats exposed to hyperthermia at 5-7 p.m., inhibition of cell division was observed only after 2 h, when MI was reduced by 2.9 times. No significant differences were found between MI in the animals of the control and experimental groups after 6 and 12 h (Fig. 1B).

Exposure of the rats to hyperthermia in the morning between 6 and 8 a.m. did not lead to inhibition of mitotic activity after 2 and 6 h (Fig. 1C). Furthermore, 2 h after the end of exposure to hyperthermia an increase was observed in the number of dividing cells by 1.7 times compared with the control. MI was reduced by 1.9 times 12 h after exposure of the rats to hyperthermia in the morning between 6 and 8 a.m. The results confirmed the presence of a circadian rhythm in the response of proliferation to the action of extremal factors at different times of the 24-h period, which the writers have observed previously [5, 6, 8, 9]. This corresponds to modern views regarding the structural and functional discreteness and the intermittent activity of function-

ing structures [4]. To ascertain the nature of inhibition of cell division during sublethal hyperthermia, an autoradiographic study was made of the number of DNA-synthesizing nuclei and the intensity of DNA metabolism, and also of the level of PM in the corneal epithelium of albino rats exposed to hyperthermia at midday between 11 a.m. and 1 p.m. These times were chosen because it is in this period that the strongest inhibition of mitotic activity was observed. The results of analysis of the circadian rhythms of the cell cycle in response to the action of the stressor will be described separately. The results of the autoradiographic studies showed that 2 h after the end of hyperthermia ILN was reduced from 6.7% in the control to 4.3% in the experiment. The decrease in the number of DNA-synthesizing cells was accompanied by a decrease in the intensity of DNA turnover. For instance, the mean number of grains of silver above the nucleus was reduced on average from 17 in the control to 11 in the animals exposed to hyperthermia. This agrees with data in the literature [9] indicating a decrease in incorporation of ^{3}H-thymidine during the first few hours after exposure of albino mice to hyperthermia for 10 min. The change in ILN 12 h after hyperthermia was not significant. Inhibition of DNA synthesis in the corneal epithelium during sublethal hyperthermia distinguished this stressor from those studied previously [9]. Another important difference between the response of the epithelium to sublethal hyperthermia and the pattern observed during hypothermia and administration of pyrogenal was an increase in the number of PM. Their number increased in the experimental group compared with the control from 3.7 to 6.3% after 2 h, from 2.7 to 9.6% after 6 h, and from 4 to 7.5% after 12 h. The principal form of PM in the cornea of the control animals was a C-mitosis and its different varieties. Exposure to hyperthermia not only led to an increase in the total number of PM, but also to a change in the spectrum of the aberrations. The number of "bridges" – a form of pathological mitosis indicating injury to chromosomes [2] – was increased in the corneas of the animals exposed to hyperthermia.

Analysis of the ratio between the phases of mitosis showed a reduction by half in the number of prophases 2 h after the end of hyperthermia. This is evidence of a decrease in the number of cells commencing mitosis. Another important feature was the presence of anaphase delay 2 and 6 h after the end of hyperthermia. The combination of anaphase delay and an increase in the number of bridges is characteristic of processes accompanied by disturbance of RNA synthesis [3, 13].

The results of these investigations confirmed previous observations [5, 8, 9] of circadian rhythms in the response of the epithelium to stressors: absence of sensitivity of the morning peak of mitoses to the inhibitory effect of the stressor, dependence of antimitotic action on the natural course of the diurnal curve of mitotic activity. At the same time, differences were found in the action of a high temperature on processes of proliferation: the possibility of a decrease in ILN and in the intensity of DNA turnover, and also an increase in the number of PM as a result of exposure of homoiothermic animals to the action of a high temperature.

LITERATURE CITED

1. N. N. Aleksandrov and S. Z. Fradkin, Hyperthermia and Hyperbaric Oxygen in the Combined Treatment of Malignant Neoplasms [in Russian], Moscow (1976).
2. I. A. Alov, The Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
3. I. A. Alov and L. S. Strochkova, Byull. Éksp. Biol. Med., No. 5, 65 (1978).
4. G. N. Kryzhanovskii, Patol. Fiziol., No. 6, 5 (1974).
5. N. B. Simon, S. S. Timoshin, and S. A. Alekseenko, Byull. Éksp. Biol. Med., No. 11, 1371 (1976).
6. N. B. Simon, S. S. Timoshin, and N. B. Murzina, Byull. Éksp. Biol. Med., No. 7, 87 (1979).
7. F. V. Sushkov and V. V. Portugalov, Byull. Éksp. Biol. Med., No. 12, 81 (1975).
8. S. S. Timoshin and S. A. Alekseenko, Byull. Éksp. Biol. Med., No. 1, 74 (1974).
9. S. S. Timoshin, N. B. Simon, and A. P. Baranov, in: Abstracts of Proceedings of the 4th International Symposium on Polar Medicine [in Russian], Vol. 2, Novosibirsk (1978), p. 146.
10. T. K. Chuiko, in: Some Problems in Mechanisms of Adaptation under Normal and Pathological Conditions [in Russian], Khabarovsk (1975), p. 132.
11. E. M. Levine and E. B. Robbins, J. Cell Physiol., 76, 373 (1970).
12. D. H. McKibben and J. L. Pecheresky, Arch. Oral Biol., 17, 291 (1972).
13. S. Pathnak and M. McGill, J. Cell Biol., 63, 258 (1974).