10. J. Halberg, E. Halberg, W. Runge, et al., Chronobiology, 1, 320 (1974).
11. W. D. Heine, E. Stöcer, and H. D. Heine, Arch. Pathol. Anat. Abt. B, Zellpathol., 9, 75 (1971).
12. H. S. Micklem, C. Asfi, N. A. Staines, et al., Nature, 227, 947 (1970).

EFFECT OF CHRONIC STRESS ON CELL DIVISION IN THE CORNEAL AND LINGUAL EPITHELIUM

E. I. Mel'nik and S. S. Timoshin

UDC 612.841.1.014.2:612.6/.06.612.591

KEY WORDS: chronic stress; mitoses; DNA synthesis.

A single exposure or five exposures to stress (moderate contact hypothermia, fixation stress, injections of pyrogenal) can inhibit mitotic activity by lengthening the G_2 phase. The level of pathological mitoses (PM), index of labeled nuclei (ILN) and the intensity of labeling (IL) were unchanged under these circumstances [7, 8]. On repeated exposure to severe stress (sublethal hyperthermia, hypoxia) DNA synthesis was activated and mitotic activity stimulated [1, 5].

The object of this investigation was to study the character of the effect of prolonged exposure to stress on epithelial proliferation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Chronic stress was simulated by cooling the animals for 1.5 h daily to 30-28°C for 28 and 35 days by the method described previously [6]. The animals were sacrificed at 4 p.m. (48 h after the last exposure to cold). The rats received an injection of [^{3}H]thymidine in a dose of 0.6 μCi/g 1h before sacrifice. The corneas were incubated for 1 h at 37°C in medium 199 with [^{3}H]-thymidine (concentration 2 μCi/ml). In one series of experiments, 2 h before sacrifice the rats were injected with colchicine in a dose of 2 μg/g, after which the index of colchicine-blocked mitoses (MIC) was determined. The mitotic index (MI), and the levels of PM, MIC, ILN, and IL were determined by the method described previously [2]. MI and MIC were expressed in promille, PM as a percentage of the total number of mitoses, and IL as the mean number of tracks above the labeled nucleus. To prove the development of a stress reaction, the animals' body weight, thymus index, and index of weight of the adrenals were determined. The adrenalin concentration in the adrenals was measured by the method in [4]. Altogether 96 animals were used in the experiments. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results showed that exposure to contact hypothermia for 28 days causes the development of a stress reaction. This was confirmed by the decrease in the animals' total body weight and the index of weight of the thymus, by an increase in the index of weight of the adrenals, and a decrease in the adrenalin concentration in the adrenals (Fig. 1). A study of cell division showed that exposure to stress for 28 days led to activation of DNA synthesis in the corneal and lingual epithelium. ILN in the cornea and tongue of the experimental animals was increased compared with the control by 1.5 and 1.4 times respectively. IL in the cornea also was increased by 1.5 times. However, neither in the tongue nor in the cornea did MI undergo any significant changes (Table 1). In rats exposed to hypothermia for 28 days, and receiving colchicine 2 h before sacrifice, preventing any change in the time of mitosis, MIC in the cornea and tongue was 2.3 and 1.8 times higher respectively than the control values. Since the experiments with colchicine were conducted asynchronously with

Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 93-94, January, 1984. Original article submitted June 9, 1983.

0007-4888/84/9701-0117$08.50

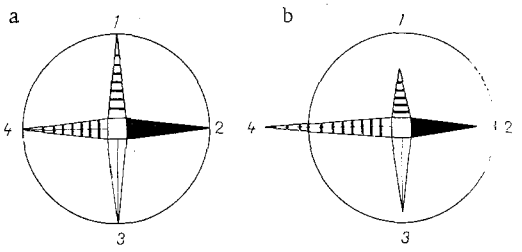

Fig. 1

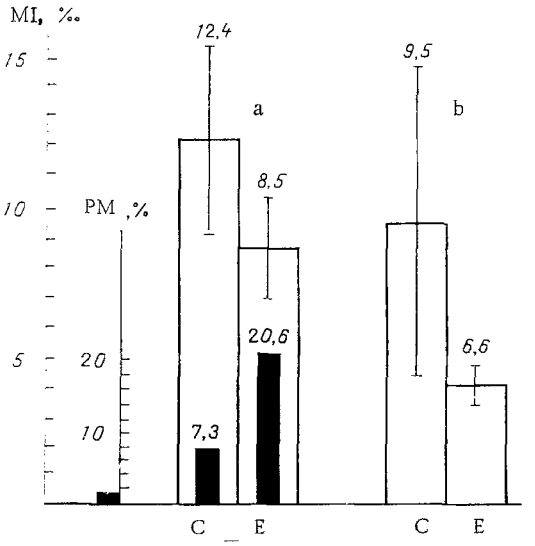

Fig. 2

Fig. 1. Parameters (in %) of the general adaptation syndrome in albino rats after contact hypothermia for 28 days. a) Control, b) experiment. 1) Adrenalin concentration in adrenals (224 μg/g), 2) animal's body weight (170 g); 3) weight index of thymus (240.7 mg/100 g); 4) weight index of adrenals (13.3 mg/100 g).

Fig. 2. Effect of 28-day exposure to stress of mitotic activity on corneal (a) and lingual (b) epithelium of albino rats (5 days after final exposure). C) Control, E) experiment.

TABLE 1. Effect of 28-Day Exposure to Stress on Cell Division in Corneal and Lingual Epithelium of Albino Rats

Group of animals	Cornea					Tongue			
	MI, ‰	PM, %	MIC, ‰	ILN, %	IL	MI, ‰	MIC, ‰	ILN, %	IL
Control	5,6±0,7	14,7±0,6	25,9±2,4	8,5±0,5	15,9±0,3	7,4±0,6	38,8±2,8	5,6±0,3	28±0,2
Experimental	6,2±0,9	12,7±0,3*	10,8±1,2*	12,5±1,0*	25,9±4,4	6,7±0,6	20,5±5,9*	7,6±0,3*	30±0,9

Legend. Here and in Table 2 asterisks indicate significance of differences compared with control.

TABLE 2. Effect of 35-Day Exposure to Stress on Cell Division in Cornea of Albino Rats

Group of animals	MI, ‰	PM,%	ILN, ‰	IL
Control	10,6±1,1	6,5±0,8	6,1±0,5	9,4±0,6
Experimental	4,0±0,6*	20,4±3*	8,6±0,9	15,7±2,2*

the previous series of experiments, it is impossible to say that in experiments without colchicine the time of mitosis was delayed, creating the illusion of equality of mitotic activity in the control and experiments. It can be tentatively suggested that this is probably one variant of the response of mitotic activity: a decrease in or normalization of MI during activation of DNA synthesis. Inhibition of mitotic activity also was observed 5 days after final 28-day hypothermia (Fig. 2), which contradicts the generally accepted view: stimulation of DNA synthesis — stimulation of mitosis. The reciprocity between increased DNA synthesis and low mitotic activity also occurred 2 days after a 35-day course of hypothermia. Whereas MI in the cornea of the experimental animals fell from 10.6 to 4.0‰, ILN increased by 1.4 times. IL increased under these circumstances by 1.7 times. Investigation of cell division 2 days after the end of 35-day hypothermia also revealed a combined increase in ILN and IL (Table 2). In this series of experiments activation of DNA synthesis accompanied an adequate increase in the number of mitoses. In all experiments

prolonged exposure to stress thus caused activation of DNA synthesis, which we regard as a structural trace of adaptation [3], whereas elevation of the PM level under these conditions we interpret as a structural trace of disadaptation [8]. Whereas in all series of experiments significant activation of DNA synthesis took place, the response of mitotic activity was characterized by several different versions: In one group of experiments reciprocity occurred between increased DNA synthesis and low mitotic activity, in the other group a high level of DNA synthesis was accompanied by normalization of mitotic activity. Finally, another variant was coincidence between the high level of DNA synthesis and the high level of mitotic activity. The presence of reciprocity between low mitotic activity and a high rate of DNA synthesis can be explained as follows: 1) activation of DNA synthesis is accompanied by lengthening of the G_2 phase or retention of the cells in it, followed by pulsed exit from that phase; 2) circadian rhythm of a response of mitotic activity to chronic stress stimulation, described by the writers previously [7]; 3) autoradiographically demonstrable activation of DNA synthesis reflects reparative DNA synthesis not ending in mitosis.

These hypotheses require experimental verification.

LITERATURE CITED

1. S. V. Vdovenko and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 8, 86 (1983).
2. T. M. Kalivetskaya and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 4, 92 (1982).
3. F. Z. Meerson, V. I. Pavlova, and G. T. Sukhikh, Patol. Fiziol., No. 5, 3 (1982).
4. V. V. Men'shikov, Methods of Clinical Biochemistry of Hormones and Mediators [in Russian], Part 2, Moscow (1974).
5. M. I. Radivoz and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 4, 98 (1982).
6. N. B. Simon and S. S. Timoshin, Byull. Éksp. Biol. Med., No. 11, 1371 (1976).
7. S. S. Timoshin, Byull. Éksp. Biol. Med., No. 2, 217 (1980).
8. S. S. Timoshin, Arkh. Patol., No. 4, 83 (1983).