EFFECT OF TRAUMATIC SHOCK ON CYTOGENETIC PROCESSES IN EPITHELIAL CELLS OF THE CORNEA AND TONGUE AND IN BONE MARROW CELLS OF ALBINO RATS

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In previous investigation the writers showed **that stress in animals with reduced powers** of adaptation (after adrenalectomy) and also stress in a form such as hyperthermia, lead to an increase in the level of pathological **mitoses** (PM) in the cornea and to inhibition of DNA synthesis [6, 9, 11]. These parameters were unchanged if the general adaptation syndrome (GAS) developed adequately [8, 10, 11].

The aim of the present investigation was to study the character of the effect of stress in the form of traumatic shock on cytogenetic processes in the cornea and bone marrow of albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on 58 male albino rats weighing 160-200 g. Traumatic shock was induced by Lindenbraten's method [3] by crushing the soft tissues of both hind limbs for 4 h. The animals were killed 3, 6, and 24 h after the end of trauma. An intraperitoneal injection of [^{3}H]thymidine in a dose of 0.6 μCi/g was given to the rats 1 h before sacrifice. Autoradiographs were prepared by the method in [8]. The mitotic index (MI) was expressed in promille. The PM level was determined only in total preparations of the cornea. A satisfactory autoradiograph could not be obtained with this does of [^{3}H]thymidine in the cornea. To rule out any possible change in the rate of mitosis, parallel experiments were carried out on animals receiving an injection of 2 μg/g colchicine 2 h before sacrifice. The mitotic index (MIC) also was determined in these animals. Preparations of bone marrow cell chromosomes were obtained by Ford's method [14]. No fewer than 50 metaphase plates corresponding to the criteria of selection were analyzed from each animal [2, 14]. To demonstrate the development of the GAS the adrenals and thymus of the rats were weighed and the concentrations of 11-hydroxycorticosterone in the blood [5], adrenalin in the liver [4], and cholesterol in the adrenals [13] were determined in the animals.

EXPERIMENTAL RESULTS

The results indicate the development of a marked stress response in the rats (Fig. 1). This was confirmed by a 1.5-fold increase in weight of the adrenals after 24 h. Activation of synthetic processes in the adrenals was shown by a fall in their cholesterol content by 1.4 times 3 and 6 h and by 1.5 times 24 h after the end of crushing. At all times of the investigation the adrenalin concentration in the liver was increased by 2.2-3.6 times and the weight of the thymus was significantly reduced. The plasma 11-hydroxycorticosterone concentration was increased by 1.6 and 2.4 times 6 and 24 h after the end of crushing, respectively.

Analysis of the mitotic cycle in the corneal epithelium revealed deep depression of cell division at all times of the investigation (Table 1). The experiments with colchicine confirmed that the decrease in weight of the dividing cells in the retina during shock did not depend on any change in the rate of mitosis. The fact that cell division in the cornea continued to be inhibited after 24 h is evidence that changes in mitotic activity during shock are not compatible with the notion of reactive inhibition, a feature of which is rapid reversibility, characteristic of G-2 delay. Inhibition of mitotic activity was accompanied by

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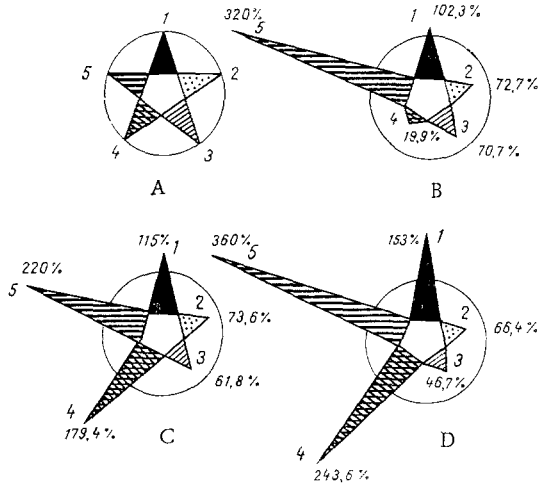

Fig. 1. Parameters of the state of the adrenals in albino rats with traumatic shock (in % of control). A) Control; B-D) 3, 6, and 24 h, respectively, after end of crushing. 1) Weight of adrenals (13.3 mg/100 g), 2) cholesterol (1.1 μmole/g), 3) weight of thymus (252.5 mg/100 g), 4) 11-hydroxycorticosterone (15.6 μg %), 6) adrenalin (0.5 μg/k).

TABLE 1. Effect of Traumatic Shock on Proliferative Processes in Epithelium of Rat Cornea and Tongue (M ± m)

Experiment conditions	Cornea			Tongue			
	MI, ‰	PM, %	MIC, ‰	MI, ‰	MIC, ‰	ILN	IL
Control	11,2±1,2	7,4±0,4	15,3±1,8	3,8±1,2	27,8±2,8	11,7±1,0	23,8±3,2
After crushing:							
3 h	1,14±0,5*	11,0±3,6	1,8±0,6*	0,9±0,1*	10,4±2,2*	5,5±0,4*	11,0±0.1*
6 h	2,4±0,4*	17,7±1,3*	3,5±1,0*	3,6±0,7	7,6±1,7*	1,5±0,2*	9,8±1,2*
24 h	3,8±1,1*	22,1±3,0*	4,8±1,6*	5,1±0,7	30,8±4,8	11.4±1,3	25,7±3.0

Legend. ILN) Index of labeled nuclei; IL) intensity of labeling. Here and in Table 2: *) $P < 0.05$ compared with control.

a significant rise in the PM level in the cornea by 2.4 and 3 times after 6 and 24 h, respectively. An increase in the number of "bridges," combined with anaphase delay, also was observed 24 h after the end of trauma. This phenomenon also is reproduced in tissue culture during inhibition of RNA synthesis [1, 15].

The number of dividing cells in the lingual epithelium was reduced by 4.2 times after 3 h. Although 6 h after the end of compression no difference was observed in MI between the control and experimental rats, the experiments with colchicine showed that this was due to the prolongation of mitosis, and in fact there was a decrease in MIC.

Autoradiographic analysis showed that shock disturbs processes of DNA synthesis. This was manifested by a decrease in the index of labeled nuclei and in the intensity of labeling 3 and 6 h after the end of crushing.

This is in agreement with data [7] on the mutagenic effect of stress. The differences after 48 h were not significant. The number of aneuploid cells showed no significant change in all the experiments. Incidentally, in the writers' previous investigations changes took place in the bone marrow cell chromosomes, including a rise in the PM level, under the influence of stress on animals with reduced powers of adaptation [12].

The results of the present experiments indicate that the disturbance of cell division processes in shock is more profound in character than the "reactive inhibition of mitosis" characteristic of the action of ordinary stressors. Prolonged depression of mitotic activity due to a disturbance of DNA synthesis, like an increase in the number of PM, are evidence of

TABLE 2. Effect of Traumatic Shock on Chromosomal Disturbances in Rat Bone Marrow Cells

Experiment conditions	No. of metaphases	Aneuploid cells, %			Cells with aberrations, %	Cells with gaps
		M	T	total		
Control	400	5,3	1,2	6,5	1	0,5
After crushing:						
24 h	350	4,6	0,8	5,4	5,1*	1,4*
48 h	350	6,3	1,1	7,4	2,3	0,6

depression of the proliferative potential under these conditions. Data showing a rise in the PM level in the cornea and also the results of metaphase analysis are evidence of the mutagenic effect of traumatic shock.

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