5. H. M. Creque, R. Langer, and J. Folkman, Diabetes, 29, 37 (1980).
6. G. L. Haberland, J. Pütter, and W. Puls, Med. Pharmacol. Exp., 14, 297 (1966).
7. M. Ishida, Y. Machida, N. Nambu, et al., Chem. Pharm. Bull., 29, 810 (1981).
8. R. Langer and J. Folkman, Am. Chem. Soc. Polymer Preprints, 18, 379 (1977).
9. N. Nelson, J. Biol. Chem., 153, 375 (1944).
10. H. M. Patel and B. E. Ryman, FEBS Lett., 62, 60 (1976).
11. H. M. Patel and B. E. Ryman, Biochem. Soc. Trans., 5, 1054 (1977).
12. M. Shichiri, R. Kawamori, Y. Goriya, et al., Acta Diabet. Lat., 15, 175 (1978).
13. M. Shichiri, R. Kawamori, Y. Goriya, et al., Endocrinol. Jpn., 23, 493 (1976).

MITOTIC INDEX IN THE CORNEAL EPITHELIUM FOLLOWING TRAUMA TO TISSUES DIFFERING IN PROLIFERATIVE ACTIVITY

A. B. Denisov

UDC 612.841.1.014.2:612.6]-063

KEY WORDS: proliferation; trauma; corneal epithelium; skin; muscle; salivary gland.

Maintenance of a certain number of cells in a tissue is an important component of homeostasis. According to many workers [8] products formed as a result of trauma are regulators of cellular homeostasis. However, despite long searches for specific growth stimulators, all substances isolated have been shown to possess relative tissue specificity [8]. Moreover, growth stimulators have been studied after trauma to strongly proliferating tissues. It is still not clear whether inactively proliferating tissues have the ability to secrete growth stimulators.

The object of this investigation was to compare the nonspecific action of trauma to tissues differing in their ability to undergo post-traumatic hyperplasia on proliferation.

EXPERIMENTAL METHOD

Experiments were carried out on 169 mature noninbred rats of both sexes. In the experiments of series I and II the rats were immobilized and the skin shaved in the dorsal region, after which the epithelium of the skin was scarified until multiple pinpoint hemorrhages appeared (area of injury about 20% of body surface). In the experiments of series III 50% of the right quadriceps femoris muscle was resected in the rats. A mock operation, with simple incision of the skin and fascia, was performed on the control rats. In the next four series of experiments about 20% by weight of the right submandibular salivary gland was removed, and in the control rats only the capsule of the gland was divided. All traumatic manipulations were carried out under pentobarbital anesthesia (50 mg/kg). Mitoses were blocked 4 h before the rats were killed by intraperitoneal injection of colchicine (3 mg/kg). The corneal epithelium (CE), which is one of the tissues with the highest level of proliferation, was chosen as test object. Furthermore, proliferation in CE is easily changed by the action of various modifying agents [1, 2, 4]. The mitotic index (MI_c) was calculated as the sum of prophases and c-mitoses (in promille) by the method described in [4]. The dose of colchicine used did not induce preprophase inhibition of mitosis and it completely blocked metaphase (the number of ana- and telophases was under 1%). In the experiments of series I and II the animals were killed on the 2nd, 3rd, 5th, and 7th days, in the remaining experiments on the 3rd day (the length of the mitotic cycle in CE cells). The results were subjected to statistical analysis [7]. Significance was assessed by the U test [3].

EXPERIMENTAL RESULTS

In both experiments trauma gave rise to reproducible and repeated changes in proliferation in CE which occurred despite the developing stress, which was reflected in atrophy of

Department of Pathological Physiology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 88-89, March, 1983. Original article submitted May 25, 1982.

0007-4888/83/9503-0359$07.50

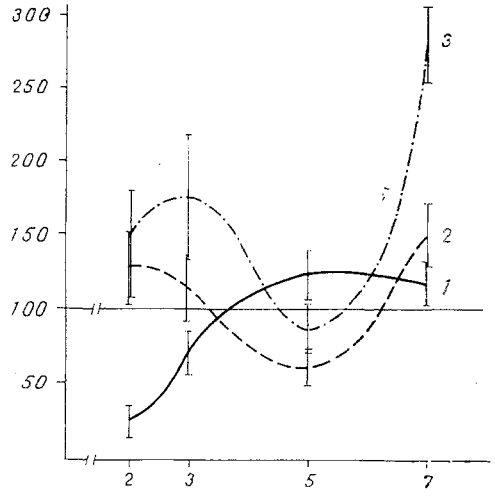

Fig. 1. Changes in MI_c in CE and in weight of thymus in rats at different times after trauma to epidermis. Abscissa, time of sacrifice (in days); ordinate, MI_c in experimental group (in % of corresponding control, M ± m). 1) Weight of thymus (in mg); 2) experiments of series I; 3) experiments of series II.

TABLE 1. Statmokinetic Index (MI_c) in CE of Rats on 3rd Day after Trauma to Muscle and Salivary Gland

Statistical index	Muscle tissue		Submandibular salivary gland							
			group 1		group 2		group 3		group 4	
	control	experiment	control	experiment	control	experiment	control	experiment	control	experiment
	1,5	3,7	2,9	6,4	3,6	11,4	12,8	35,8	22,3	45,1
	2,6	4,8	4,0	6,5	3,7	11,4	15,1	37,4	23,9	45,9
	2,9	4,9	4,4	9,9	5,4	14,4	20,3	41,3	30,2	48,2
	3,5	7,7	6,4	10,2	5,7	16,4	31,0	45,3	38,0	49,0
	7,8	8,1	6,6	10,3	6,6	17,4	39,0	45,5	42,4	60,2
	8,7	9,4	—	14,2	7,1	17,5	40,0	50,7	42,9	62,7
	9,2	10,6	—	—	8,4	17,5	—	53,7	45,1	66,0
	10,4	12,4	—	—	11,8	18,7	—	54,8	49,0	76,9
	11,2	12,4	—	—	12,4	24,3	—	55,4	—	—
	11,3	13,5	—	—	—	24,4	—	56,4	—	—
	—	16,5	—	—	—	—	—	63,5	—	—
M	6,9	9,4	4,9	9,6	7,2	17,3	26,4	49,1	36,7	56,8
$\pm m$	1,2	1,2	0,7	1,2	1,0	1,4	4,9	2,6	3,6	4,1
V	56	44	33	30	44	26	45	18	27	20
$\pm m_V$	6	4	6	5	5	2	8	1	3	2
A	2,70	2,87	1,60	1,86	2,51	2,70	1,86	2,87	2,31	2,31
$\pm m_A$	0,68	0,66	0,91	0,84	0,71	0,68	0,84	0,66	0,75	0,75
$t=A/m_A \geqslant 3,0$	3,97	4,35	1,75	2,21	3,53	3,97	2,21	4,35	3,08	3,08
E	5,10	6,10	0,20	1,17	4,11	5,10	1,17	6,10	3,12	3,12
$\pm m_E$	0,75	0,76	0,25	0,60	0,54	0,75	0,60	0,76	0,71	0,71
$t=E/m_E \geqslant 3,0$	6,80	8,02	0,80	1,95	7,61	6,80	1,95	8,02	4,39	4,39
$M_0/M_K \cdot 100\%$	136		196		240		186		155	
P	<0,05		0,01		<0,001		<0,001		0,001	

the rats' thymus (Fig. 1). The biphasic trend of the changes in the level of proliferation reflected the course of regeneration in the injured skin: The first peak of increased proliferation coincided with activation of cell proliferation at the edge of the wound, whereas the second peak was connected with intercalated growth outside the wound [6].

Statistical analysis of the results of the subsequent experiments showed considerable scatter of values in the groups around a mean value, high asymmetry, and positive excess, significant in nearly all cases, in the groups (Table 1). The difference in the values of MI_c in the control animals was due to the effect of circadian and seasonal biorhythms, for individual experiments were carried out at different times of the year and day. Accordingly, the nonparametric U test was used to calculate significance.

Trauma to muscle was shown to stimulate mitosis with an increase in MI_c (by 36%) in CE. By performing four identical experiments with injury to the submandibular salivary gland it was shown that the phenomenon of stimulation of proliferation in CE after trauma is reliably reproduced.

Acute injury to epithelial, muscle, and gland tissues had a stimulating action on proliferation of CE with the participation of distant mitogenic substances, for contact between CE cells and products produced by tissue trauma was effected entirely by the humoral route. This effect probably does not depend on the ability of individual tissues to undergo post-traumatic proliferation, established during evolution [9]. However, the fact that stimulation of MI_c varied from 155 to 240% is evidence of the action of other, as yet unstudied factors, on proliferation. It can be tentatively suggested that the phenomenon studied is connected with mediators of the autonomic nervous system, whose concentration rises rapidly after any kind of trauma, and which participate in the mechanism of action of growth stimulators [5]. This is shown by the nonspecificity of the response: In the chosen tissues both the number of mitoses after trauma and the times of maximal increase in the level of proliferation differed.

The results as a whole are evidence that proliferation in CE is stimulated by trauma to rapidly and slowly proliferating tissues, and this is evidently connected with the production of remotely acting substances.

LITERATURE CITED

1. G. A. Vinogradova, Byull. Éksp. Biol. Med., No. 11, 105 (1960).
2. M. T. Gololobova, Byull. Éksp. Biol. Med., No. 9, 96 (1962).
3. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973).
4. A. B. Denisov, Patol. Fiziol., No. 5, 84 (1976).
5. A. B. Denisov and V. V. Mikhailov, Byull. Éksp. Biol. Med., No. 3, 364 (1981).
6. E. A. Efimov, Post-traumatic Regeneration of the Skin [in Russian], Moscow (1975).
7. G. F. Lakin, Biometrics [in Russian], Moscow (1980), p. 117.
8. L. K. Romanova, in: Advances in the Study of Regeneration [in Russian], ed. L. D. Liozner, Moscow (1977), p. 269.
9. D. S. Sarkisov, Outlines of the Structural Bases of Homeostasis [in Russian], Moscow (1977).

EFFECT OF EXOGENOUS CALMODULIN ON LYMPHOCYTE PROLIFERATION IN NORMAL SUBJECTS

L. V. Gorbachevskaya, T. V. Borovkova, V. O. Rybin, and N. A. Fedorov

UDC 616.112.94.612.6/-06:612.398.612.015.31:546.41

KEY WORDS: proliferation; lymphocytes; calmodulin.

Calmodulin (CM) is a low-molecular-weight calcium-binding protein which mediates the effect of Ca^{++} on several metabolic processes (synthesis and breakdown of cyclic nucleotides, glycogenolysis, lipolysis, etc.), and also its effect on membrane permeability (activation of Ca—ATPase of human erythrocytes, increased permeability of synaptic vesicles for mediators, and so on) [6]. This protein is found in virtually all animal tissues. Its polyfunctionality suggests that it is one of the principal intracellular receptors for Ca^{++} ions.

Ca-binding proteins are known to possess Ca-ionophore properties, i.e., they can increase the passive permeability of membranes for Ca^{++}. Lymphocytes react by an increase in blast transformation to many agents, including those which increase membrane permeability for Ca^{++} ions [5].

Department of Functional Diagnosis and Clinical Radiology, Moscow Research Institute of Tuberculosis, Ministry of Health of the RSFSR.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 90-91, March, 1983. Original article submitted June 2, 1982.