There is as yet no generally accepted theory to account for differences in the response of cell populations to changes in ambient temperature conditions over a wide range of temperatures suitable for mitosis [6]. Without going into a detailed discussion of this problem, it may be simply pointed out that the results obtained for the duration of the mitotic cycle of Chinese hamster cells at 30-39°C in the present experiments show that the presynthetic period makes the main contribution to the sharp increase in the duration of interphase at the "critical" temperature.

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CHANGES IN MITOTIC ACTIVITY IN THE CORNEAL EPITHELIUM OF RATS OF DIFFERENT AGES AFTER NOCICEPTIVE STIMULATION

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UDC 612.841.1.014.3:612.6]:612.884

The effect of nociceptive stimulation on mitotic activity in the corneal epithelium was investigated in 21-day-old rat fetuses and in rats aged 3, 4, 5, 7, 10, 15, 20, and 25 days. Mitotic activity was not significantly changed 45 min after nociceptive stimulation of the animals (amputation of one-third of the tail) in the cornea of the fetuses and day-old rats. Between the 3rd and 10th days of postnatal development reactive inhibition of mitosis in response to nociceptive stimulation was gradually formed. After 10 days this response was intensified and reached a maximum by the 25th day. Reactive inhibition of mitotic activity is connected with delayed entry of the cells into mitosis.

KEY WORDS: *Corneal epithelium; nociceptive stimulation; mitotic index.*

Numerous investigations have shown that in response to the action of various stressors (electric shock, nociceptive stimulation, and so on) reactive inhibition of mitosis is observed in the ectodermal tissues of mammals [4-11]. Adrenalin secreted by the adrenals during excitation of animals has been shown to play in important role in the mechanism of this response. Meanwhile, other investigations have shown the absence of reactive inhibition of

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1367-1369, November, 1976. Original article submitted April 23, 1976.

TABLE 1. Mitotic Activity of Corneal Epithelium of Rats of Different Ages under Normal Conditions and after Nociceptive Stimulation

Age, days	Group of rats	Mean MI, ‰	P	Mean number of mitoses	Phase of mitosis (mean values)		
					EP + P	M	A + T
1	Control	6,3	0,30	46,4	8,4	15,4	22,6
	Experimental	5,2		34,3	4,8	13,0	16,5
3	Control	13,8	0,002	114,2	10,2	39,6	64,4
	Experimental	7,6		61,0	3,4	10,6	47,0
4	Control	6,9	0,60	62,5	17,7	19,8	25,0
	Experimental	6,7		60,2	8,0	16,4	35,8
5	Control	9,0	0,02	83,9	6,2	24,8	52,9
	Experimental	5,1		48,2	1,2	4,0	43,0
7	Control	6,9	0,15	66,0	2,8	12,4	50,8
	Experimental	5,0		45,4	4,6	5,4	35,4
10	Control	7,5	0,02	79,1	15,4	35,7	28,0
	Experimental	3,7		36,3	2,8	6,7	26,8
15	Control	7,4	0,001	105,0	29,2	16,4	59,4
	Experimental	2,2		28,6	2,2	3,5	22,9
20	Control	10,1	0,001	156,6	21,4	59,0	76,2
	Experimental	4,4		68,6	2,3	11,7	54,6
25	Control	21,5	0,001	270,2	34,6	120,3	115,3
	Experimental	4,7		72,9	5,0	12,3	55,6
Fetuses	Control	13,3	0,07	77,3	21,1	29,0	27,2
	Experimental	11,4		80,0	13,0	31,4	35,6

Legend. EP) early prophases, P) prophases, M) metaphases, A) anaphases, T) telophases.

mitosis in the tissues of fetal and newborn mice and rats; they exhibit a capacity for such inhibition only at a certain stage of postnatal development [5, 9].

The time of appearance of a proliferative response of the tissue to stressor action in ontogeny and changes in its pattern at different periods of life of the animals were investigated.

EXPERIMENTAL METHOD

The effect of nociceptive stimulation on mitotic activity in the corneal epithelium was investigated in rats of the following ages: 1, 3, 4, 5, 7, 10, 15, 20, and 25 days. For each age group there were on average five experimental and five control rats taken from the same litter. The nociceptive stimulation applied to the experimental animals consisted of amputation of one third of the tail. Intact rats served as the control. The rats were killed 45 min after experimental stimulation. Similar nociceptive stimulation also was applied to pregnant rats 1-2 days before birth of the young. These rats were killed 45 min later and the fetuses were taken from the urterine cornua. Fetuses taken from intact rats served as the control for this group. Total preparations of the cornea were stained by Carazzi's hematoxylin. In each case 100 fields of vision of the microscope were examined. The mitotic index (MI) was expressed in promille. It was calculated for the cornea as a whole and also for its peripheral and central zones separately. The phases of mitosis were taken into account. All the experiments were performed at the same time of day, namely from 5 to 8 p.m., at a time when the level of mitotic activity in the cornea does not change substantially and remains fairly high. The experimental results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

The results show that 45 min after nociceptive stimulation of the pregnant rats the number of mitoses in the cornea of the fetuses was reduced, but not significantly ($P = 0.07$). The response to experimental stimulation differed in different zones of the cornea: In cells of the peripheral zone the mean value of MI was reduced ($P = 0.02$), whereas in cells

of the central zone there was no response (MI in the cornea of the experimental fetuses was 11.1 °/₀₀ and of the controls 11.9 °/₀₀).

No significant changes in the level of mitotic activity in response to nociceptive stimulation were found in the corneal epithelium of the rats aged 1 day.

By contrast, in the experimental rats aged 3 days there was a definite decrease in the mean value of MI: by 45% in the epithelium of the cornea as a whole ($P = 0.02$), by 39% in its peripheral zone ($P = 0.005$), and by 50% in the central zone ($P = 0.01$). This decrease was accompanied by a sharp reduction in the number of early phases of mitosis.

In the group of rats aged 4, 5, and 7 days reactive inhibition of mitotic activity caused by nociceptive stimulation was not yet stable, for it was not present in animals aged 4 and 7 days ($P = 0.15$), although it was clearly manifested in the 5-day-old rats ($P = 0.02$). In the experimental rats aged 5 days there was a sharp decrease (more than fivefold) in the number of early prophases, prophases, and metaphases.

In the rats aged 10 days nociceptive stimulation led to sharp reactive inhibition of mitosis. The decrease in the mean value of MI in the epithelium of the whole cornea was 51%, in the peripheral zone 52%, and in the central zone 47%. It was accompanied by a sharp decrease in the number of all phases of mitosis to anaphase inclusive.

In all subsequent age groups, i.e., in rats aged 15, 20, and 25 days, a considerable decrease in the mean value of MI was observed after nociceptive stimulation. With increasing age this response became stronger. It reached its maximal values in rats aged 25 days, in the cornea of which the number of mitoses decreased by 78%. In both zones of the cornea the reaction of inhibition of mitosis was clearly marked. The total decrease in MI was due to a particularly sharp reduction in the number of early prophases, prophases, and metaphases (by 7 to 15 times depending on age).

These results show that the formation of reactive inhibition of mitosis in the corneal epithelium of rats in response to nociceptive stimulation takes place between the 3rd and 10th days of postnatal development. These results are close to those obtained by Suvorova [9], who found that the reaction of inhibition of cell division in the corneal epithelium of mice after electrical stimulation is formed between the 6th and 14th days of postnatal development. The absence of inhibition of mitosis in rats under 3-days-old in the present experiments and the unstable character of its manifestation in animals under the age of 10 days may perhaps be explained by the inadequate maturity of the neuroendocrine factors lying at the basis of this reaction or inability of the tissues of young animals to respond by inhibition of mitotic division to nociceptive stimulation [9]. In all cases in which the decrease in MI caused by nociceptive stimulation was clearly marked, it was accompanied by a reduction in the number of the early stages of division, evidence of delay in the entry of the cells into mitosis. In the postnatal period of development of the rats the reaction of the epithelium of the peripheral and central zones of the cornea to nociceptive stimulation was similar in character. Changes in MI in each zone correlated with changes in MI in the cornea as a whole.

Since during nociceptive stimulation of the animal the functional activity of the adrenal is increased and it discharges an increased quantity of adrenalin into the blood stream, it was interesting to compare these results with those relating to the action of exogenous adrenalin on proliferative processes.

The writer showed previously [2] that 45 min after injection of adrenalin there is a significant increase in the number of cell divisions in the cornea of rats aged 3 and 4 days. However, it was found that this increase is caused not by stimulation of the entry of the cells into mitosis but by delay in the passage of the cells mainly through the middle and late stages of division. The antimitotic action of the hormone at this period of the investigation begins to be manifested particularly clearly in animals toward the age of 70 days. Consequently, the distinguishing feature of the action of adrenalin on cell division is that it not only inhibits the entry of the cells into mitosis, but it also changes the duration of mitosis.

In young animals, by contrast with adults, 45 min after nociceptive stimulation complete correlation is not observed between the action of exogenous adrenalin and of nociceptive stimulation on proliferative processes in the corneal epithelium. This suggests that

in the early stages of ontogeny the response of the animals to different types of stimuli (hurmoral—adrenalin and reflex—pain) may differ in character.

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DIURNAL CHANGES IN THE NUMBER OF MITOSES AND OF DNA-SYNTHESIZING CELLS IN TISSUES OF YOUNG RATS

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UDC 612.014.3:612.6"52"

Regular diurnal changes in the number of mitoses (MI) and the number of DNA-synthesizing cells (ILN) were demonstrated in the liver, epidermis, and exogenous part of the pancreas of rats aged 7 days. The character of these changes differed in the various tissues. No regular correlation was found between diurnal changes in MI or ILN.

KEY WORDS: *Mitotic index (MI); index of labeled nuclei (ILN); diurnal changes in MI and ILN; liver; epidermis; pancreas.*

The study of the character of the diurnal rhythm of DNA-synthesizing cells is important in order to determine the time of most effective administration of inhibitors or stimulators of cell division which act mainly on the premitotic stage of DNA synthesis [2]. This study is also interesting as a means of shedding light on age changes in the character of the rhythm of cell division.

As yet this problem has been studied virtually entirely in tissues of adult animals [4, 5, 6-11], whereas in young animals, distinguished by both the pattern of their diurnal rhythm of mitosis and the level of cell proliferation, it remains almost completely unstudied. The investigation described below was accordingly carried out.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1369-1371, November, 1976. Original article submitted April 2, 1976.