

EFFECT OF HYDROCORTISONE ON REPRODUCTION OF RAT CORNEAL EPITHELIAL CELLS AT VARIOUS PERIODS OF POSTNATAL ONTOGENESIS

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After injection of hydrocortisone into rats of different ages (1-168 days) a decrease in the number of cell divisions was found in the corneal epithelial. The inhibitory action of the hormone began after 4 h, and it continued for the next 8 h.

Among the hormones participating in the regulation of cell division in the body, an important role is played by the glucocorticoid adrenocortical hormones, which reduce the number of cell divisions in the tissues of adult animals [1, 4, 5, 7-12]. The antimitotic action of the glucocorticoids has been shown to be highly selective, for they reduce the number of mitoses sharply in lymphoid tissues but to a lesser degree in epithelial tissues. The action of cortisone and adrenalin on cell proliferation has also been shown to depend on the age of the experimental animals, which raises the question of the formation of tissue reactions to hormones with respect to cell proliferation in the course of the individual development of animals [3, 5, 6].

The objective of the present investigation was to study the action of hydrocortisone on the mitotic activity of cells of the stratified squamous corneal epithelial of albino rats during their postnatal ontogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of 14 age groups. Each group consisted of 5-6 control and 5-6 experimental animals. Hydrocortisone was injected intraperitoneally in a dose of 5 mg/100 g body weight in 10 h. Control animals received the corresponding volumes of physiological saline. The corneas were fixed in Carnoy's fluid and total two-dimensional specimens were obtained and stained with Mayer's hematoxylin. Cells and mitoses (binocular attachment, objective 90, ocular 7) were counted along mutually perpendicular lines passing through the center of the cornea, while mitoses and cells were counted separately at the center and at the periphery. In each case between 12,000 and 24,000 cells were examined. The mitotic index (MI) was calculated in promille. The results were analyzed statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

Experiments of Series I. The effect of hydrocortisone on MI of the corneal epithelial of the rats of different age groups was studied in this series. The animals were sacrificed 5 h after injection of the hormone. The results are given in Table 1.

In the control animals changes in the total MI were observed in the course of postnatal ontogenesis (Table 1). On the 1st day after birth comparatively high mitotic activity was found in the cornea of the rats, but on the 3rd-9th days the level of cell proliferation fell ($P=0.001$). Starting from the 11th day a further

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TABLE 1. Changes in MI (in ‰) of Corneal Epithelial Cells in Animals of Different Ages after Receiving Injection of Hydrocortisone

Age of animals (in days)	MI - total			MI - center			MI - periphery		
	con-trol	expt.	P	con-trol	expt.	P	con-trol	expt.	P
1	7,7	3,1	0,0001	8,0	3,3	0,0001	7,4	2,6	0,0001
3	3,5	1,6	0,0001	3,5	2,0	0,002	3,5	1,4	0,0001
5	4,2	2,2	0,0001	4,1	2,6	0,003	4,2	2,0	0,0001
7	5,6	2,5	0,0001	5,8	3,0	0,003	5,4	2,3	0,0001
9	3,9	1,9	0,0001	4,1	2,4	0,002	3,8	1,6	0,0001
11	8,2	3,9	0,0001	8,2	4,4	0,001	8,7	3,7	0,0001
14	8,3	10,4	0,062	8,2	8,7	0,814	8,2	11,4	0,026
17	19,5	7,5	0,0001	19,2	8,2	0,0001	19,8	7,2	0,0001
21	13,8	6,1	0,0001	13,6	6,0	0,0001	14,0	6,1	0,0001
28	10,7	2,7	0,034	10,5	3,2	0,0001	10,9	2,3	0,0001
35	7,1	5,5	0,971	6,9	5,0	0,258	7,3	5,8	0,442
56	7,3	4,3	0,019	7,4	3,8	0,01	7,3	4,2	0,041
112	13,2	5,6	0,0001	12,5	5,4	0,0001	13,5	5,6	0,0001
168	13,3	7,1	0,001	11,7	7,2	0,013	14,1	7,1	0,0001

TABLE 2. Changes in MI (in ‰) of Corneal Epithelial Cells of Rats Aged 3 Days at Different Times after Injection of Hydrocortisone

Time after injection (in h)	MI - total			MI - center			MI - periphery		
	con-trol	expt.	P	con-trol	expt.	P	con-trol	expt.	P
1	7,8	7,2	—	8,5	8,0	—	7,3	5,0	—
2	6,7	5,7	—	6,5	6,0	—	6,8	5,6	—
3	6,8	6,7	—	6,8	7,1	—	6,8	6,5	—
4	6,6	2,9	0,001	6,9	3,4	0,001	6,7	2,8	0,002
5	7,1	3,6	0,001	7,6	4,0	0,008	6,5	3,4	0,05
7	8,6	4,7	0,034	9,4	5,1	0,038	8,2	4,5	0,009
9	9,6	5,6	0,001	9,5	5,7	0,003	8,9	5,5	0,001
12	10,0	4,9	0,013	11,8	5,2	0,023	8,0	4,8	0,001
16	5,1	4,3	—	5,4	4,9	—	5,0	3,9	0,041

increase in mitotic activity began, and this reached its maximum on the 17th day ($P=0.0001$). This increase in the number of mitoses coincided with the period of opening of the palpebral fissure, which takes place in rats at the age of 14–16 days [2]. In the animals of the following age groups, MI of the corneal epithelial again fell until the 56th day of life. After the rats had reached sexual maturity, which in rats is at the age of 2 months, the level of mitotic activity in the cornea increased again, but to a lesser degree than in the 17-day animals. The changes in MI were similar at the periphery and in the center of the cornea.

After injection of hydrocortisone there was a significant decrease in the total MI in all groups investigated except in rats aged 14 and 35 days.

Moreover, in the experimental animals aged 14 days the number of cell divisions in the peripheral part of the cornea was increased.

Experiments of Series II. Changes in mitotic activity in the corneal epithelial of rats at various times after administration of hydrocortisone were studied in these experiments.

Animals aged 3 and 28 days were sacrificed at different times after injection of the hormone. The results are given in Tables 2 and 3.

As Table 2 shows, 4 h after injection of hydrocortisone the total number of mitoses in the corneal epithelial began to decrease, as also did the number in the center and at the periphery. The inhibitory action of the hormone lasted for the next 8 h, and after 16 h no differences could be found between the number of mitoses in the control and experimental animals. On the other hand, these results indicate the similar character of the action of hydrocortisone on mitotic activity in the various zones of the corneal epithelial of rats aged 3 days.

The significant decrease in the total MI of rats aged 28 days (Table 3), just as in those aged 3 days, was observed 4 h after injection of the hormone, and it lasted for the next 8 h. However, the inhibitory action of hydrocortisone in the central part of the corneal epithelial was found only 7 h after the injection, whereas at the periphery of the cornea the inhibitory action of hydrocortisone became apparent after 4 h.

TABLE 3. Changes in MI (in %) of Corneal Epithelial Cells of Rats Aged 28 Days at Various Times after Injection of Hydrocortisone

Time after injection (in h)	MI - total			MI - center			MI - periphery		
	con-trol	expt.	P	con-trol	expt.	P	con-trol	expt.	P
1	18,8	17,7	—	20,2	18,9	—	18,1	17,2	—
2	10,0	10,6	—	10,8	10,8	—	9,6	10,5	—
3	9,5	9,4	—	8,5	9,4	—	10,0	9,2	—
4	5,0	3,0	0,029	4,7	3,2	0,065	5,2	3,0	0,018
5	10,1	6,2	0,017	10,4	6,9	0,199	10,0	5,9	0,009
7	9,9	5,7	0,005	9,4	5,6	0,002	10,3	5,7	0,008
9	4,2	1,3	0,007	4,2	1,0	0,006	4,0	1,4	0,011
12	16,4	7,1	0,006	16,7	4,8	0,004	16,3	8,3	0,023
16	10,4	7,8	0,141	10,1	8,1	—	10,5	7,7	—

Hence, the inhibitory action of hydrocortisone on the total MI in the corneal epithelial appeared 4 h after its injection. It is impossible to pinpoint the exact phase of the mitotic cycle at which hydrocortisone acts, but it most probably influences the premitotic phase of the cycle.

The results indicate that mitotic activity in the corneal epithelial, if determined at the same time of day, undergoes significant changes in the course of postnatal ontogenesis. The absence of a decrease in MI in the animals aged 14 and 35 days after injection of hydrocortisone suggests differences in the degree of sensitivity of the corneal epithelial cells at different age periods to this hormone. The possibility is not ruled out that one possible cause of these results could be the existence of periods during the 24 hours in which the sensitivity of the cells to hydrocortisone is reduced or increased.

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