

AGE CHANGES IN EFFECT OF HYDROCORTISONE ON MITOTIC ACTIVITY OF RAT THYMUS LYMPHOCYTES

É. T. Ostroushko

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The time of appearance of the antimitotic action of hydrocortisone on lymphocytes of the thymus cortex in rats depends on the age of the animals. In young rats aged 1-9 days a decrease in the number of mitoses occurs after 5h, but in rats aged 14 and 28 days it occurs after 2 h.

The glucocorticoid hormone of the adrenal cortex reduce the number of cell divisions in tissues [1, 3, 8-12]. It has also been found that the degree of inhibitory action of hormones on cell proliferation differs in different tissues. The largest decrease in the number of mitoses was observed in the lymphoid organs, especially the thymus. These investigations were carried out on adult animals. With regard to the other adrenal cortical hormone (adrenalin), its action on mitotic activity, even of the same tissue, has been shown to depend on the age of the experimental animals [2, 5]. These results suggest that tissue responses to the action of hormones on cell division are established in the process of individual development of animals.

The object of this investigation was to study the effect of hydrocortisone on mitotic activity of thymus lymphocytes of albino rats at different ages.

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. The control animals received corresponding volumes of physiological saline at the same times. The thymus was fixed in Carnoy's fluid and histological sections, 5-6 μ in thickness, were stained with hematoxylin. Cells and mitoses (binocular attachment, 900 \times) were counted in the cortical layer of the thymus, in each case during examination of 20,000-25,000 cells. The mitotic index (MI) was calculated in promille. Statistical analysis of the results was by the Fisher - Student method.

EXPERIMENTAL RESULTS

Experiments of Series I. Investigation of Effect of Hydrocortisone on MI of Rat Thymus at Different Ages

In all series of experiments the hormone was injected at 10 A.M. and the animals sacrificed at 3 P.M. The results obtained in series I are shown in Table 1.

The results in Table 1 show that age changes in mitotic activity occurs in the control animals. In the neonatal period, lasting until the age of 5 days, a relatively high mitotic activity was observed, but then until the age of 17 days the mitotic activity gradually fell, subsequently remaining at about the same level.

A significant decrease in MI under the influence of hydrocortisone took place only before the age of 9 days. In later age groups under the same experimental conditions, no significant decrease in the number

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TABLE 1. Changes in MI of Thymus Lymphocytes After Injection of Hydrocortisone Depending on Age of Rats

Group of animals	Age (in days)													
	1	3	5	7	9	11	14	17	21	28	35	56	112	168
Control	10,4	12,0	8,2	6,9	5,9	5,1	5,0	4,9	4,0	4,0	3,6	4,0	5,0	3,1
Experimental	6,0	5,9	6,2	3,8	3,7	5,2	5,1	5,8	4,6	5,1	4,2	4,2	5,2	3,2
P	0,001	0,0001	0,009	0,005	0,019	—	—	0,07	0,13	0,51	0,58	0,85	—	—

TABLE 2. Changes in MI of Thymus Lymphocytes in Rats of Different Ages 1 and 2 h After Injection of Hydrocortisone

Age (in days)	Time after injection of hydrocortisone (in h)					
	1			2		
	con- trol	expt.	P	con- trol	expt.	P
3	8,2	7,2	0,15	4,8	4,1	0,30
9	7,5	6,0	0,35	6,2	5,8	0,70
14	4,6	4,6	—	3,7	2,6	0,03
28	4,3	4,2	—	3,2	2,1	0,005
35	3,6	4,2	0,07	2,6	2,6	—
56	5,6	5,7	—	7,8	6,3	0,50
75	4,1	2,8	0,13	2,5	4,1	0,07

TABLE 3. Changes in MI of Thymus Lymphocytes of Rats Aged 3 and 28 Days at Different Times After Injection of Hydrocortisone

Time after injection of hydrocortisone (in h)	Time of day	Age (in days)					
		3			28		
		con- trol	expt.	P	con- trol	expt.	P
1	11	6,8	8,6	0,35	5,4	5,5	—
2	12	5,4	4,2	0,26	4,7	3,1	0,02
3	13	7,3	6,5	0,23	8,0	5,2	0,08
4	14	7,1	7,6	0,44	7,4	5,9	0,09
5	15	11,0	9,0	0,04	4,4	4,5	0,77
7	17	10,5	7,7	0,01	7,2	6,3	0,50
9	19	7,1	6,6	0,70	6,1	4,5	0,03
12	22	9,7	6,3	0,02	6,0	1,5	0,0001
16	2	5,5	1,0	0,001	5,7	0,9	0,0001

of mitoses was found. In the experiments of series II and III the effect of the compound was studied on animals of different ages at different times after its injection.

Series II. Changes in Mitotic Activity of Thymus Lymphocytes at Short Periods after Injection of Hydrocortisone

The results obtained in series II are given in Table 2. No decrease in the number of mitoses was found 1 h after injection of the hormone in the animals of any age groups. A decrease in the number of mitoses 2 h after injection was found only in animals aged 14 days (close to significant, $P=0.03$) and aged 28 days (statistically significant, $P=0.005$). These results indicate a higher degree of sensitivity of the thymus lymphocytes in rats at these ages compared with other age groups.

The object of these experiments, in which animals of two age groups (3 and 28 days) were sacrificed at different intervals of time after injection of the hormone, was to study the time of appearance of the inhibitory action of hydrocortisone. The results are given in Table 3.

In rats aged 3 days hydrocortisone began to exert its inhibitor action on mitosis 5 h after injection. This effect reached a maximum after 7 h. However, 9 h after injection no difference was found in the number of mitoses in the control and experimental animals, but later the inhibitory action of the hormone began to show itself once again. These results indicate that changes in cell division after a single injection of the hormone occur in two phases. A special investigation is necessary to establish the causes of this phenomenon.

The decrease in MI in rats aged 28 days, as in the experiments of series II, was observed after 2 h. This followed by a period (from 3 to 7 h) during which no decrease in the number of mitoses was found in the experimental rats. However, considerable inhibition of cell division was found 9 h after injection, and this persisted until the end of the observations. Consequently, in this case also, the results obtained indicated that changes in cell division after injection of the hormone occur in two phases.

The problem of the mechanism of action of glucocorticoid hormones on mitotic cell division has not yet been adequately explained. The results described above show that the inhibitory action of hydrocortisone does not appear sooner than 2 h after its injection. This means that the hypothesis of the inhibitory

action of hydrocortisone on passage of cells from the premitotic phase of the mitotic cycle into mitosis must be treated with some reservation.

The existence of two phases of changes in the number of cell divisions after administration of the hormone suggests that the antimitotic action of hydrocortisone possess different mechanisms. It has recently been postulated [7] that hydrocortisone may influence intracellular molecule rearrangements and the permeability of cell membranes, thereby influencing exchanges between the cells and the surrounding medium. The possibility is not ruled out that the biphasic nature of changes in the number of mitoses may be the result of the nonsimultaneous action of the hormone on these different processes in the cell.

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