DIURNAL CHANGES IN MITOTIC ACTIVITY IN THE EPITHELIUM AND STROMA OF THE THYROID AND PARATHYROID GLANDS OF YOUNG RATS

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Investigations of cell division in the thyroid and parathyroid glands of young rats showed the existence of diurnal rhythms of mitosis, whose phase dynamics has organ and tissue specificity and differs from that of adult rats, in these organs. The diurnal rhythm of mitosis is identical in the cells of follicles of different sizes, but mitotic activity is reduced in the large follicles.

Little information is available on changes in the number of mitoses during the 24-h period in the thyroid epithelium of rats. According to Hunt [5], the number of mitoses in the thyroid epithelial cells of adult animals reaches a minimum at 8 a.m. and a maximum at 11 p.m. Conversely, Mühlemann et al. [6, 7] consider that the highest mitotic activity of the thyrocytes of these animals occurs at 7 a.m. and the lowest at 10 p.m. It is difficult to compare the results of these investigations, for they were carried out after long intervals of time (8-12 h). No information could be found in the literature on changes in the mitotic activity in the thyroid gland of young rats during the 24-h period.

In an earlier investigation conducted at 3-h intervals a biphasic diurnal rhythm of mitosis in the epithelium and a monophasic rhythm of mitosis in the connective-tissue cells were found in the thyroid gland of adult rats. A diurnal rhythm of mitotic activity in the epithelium of the parathyroid gland also was demonstrated in these animals [2-4].

In the present investigation the mitotic activity in the epithelium and stroma of the thyroid gland and in the epithelium of the parathyroid glands of young rats was studied during the 24-h period. During investigation of the reproduction of the thyroid epithelial cells their arrangement in the follicles of different sizes and in the interfollicular complexes was taken into account so that any dependence of proliferation of the thyrocytes on their differentiation and development could be assessed.

TABLE 1. Mean Diurnal Folliculogram and Cytogram of the Thyroid Gland in Young Rats

	Class of follicles				
	1	2	3	4	5
No. of follicles (%)	39,0	58,3 P_1=0,0001	1,9 P_{1-2}=0,001	0,5 P_{1-3}=0,001	0,3 P_{1-3}=0,001
No. of follicular cells (%)	14,2	63,0 P_1=0,0001	12,5 P_2=0,0001	7,5 P_{1-3}=0,001 P_2=0,0001	2,8 P_{1-2}=0,001 P_3=0,002 P_4=0,050

Note. The subscript attached to P indicates the class of follicles with which the result is compared.

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TABLE 2. Height of Follicular Cells in Thyroid Gland of Young Rats during the 24-h Period

Time of day	Height of cells (μ)
10	8,0
13	8,3
16	8,4
19	8,6
22	8,1
1	9,0
P_{10}=0,013 P_{22}=0,026	
4	8,4
7	8,5
Mean for 24 h	8,5

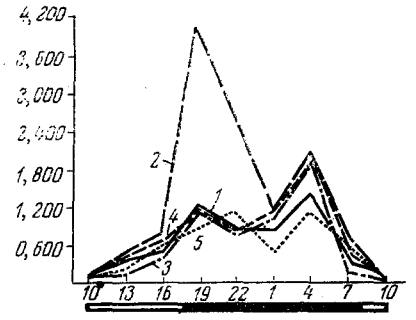

Fig. 1. Changes in mitotic indices in thyroid epithelium of young rats during the 24-h period. Here and in Figs. 2 and 3: abscissa, time of day (in h); ordinate, mitotic index (in ‰). 1) MIFC; 2) MIIFC; 3) MI_1; 4) MI_2; 5) MI_3.

EXPERIMENTAL METHOD

Experiments were carried out on 48 young male albino rats (mean weight 31 g, age about 20-35 days) receiving a natural diet and kept in a natural alternation of daylight and darkness (February). The animals were killed 6 at a time at 10 a.m., 1, 4, 7, and 10 p.m., and 1, 4, and 7 a.m. The thyroid and parathyroid glands were fixed in Zenker-formol with acetic acid and sections 6 μ in thickness were stained with hematoxylin-eosin. The total mitotic index (TMI) of the epithelial cells of the thyroid was calculated for 60,000-70,000 thyroctyes. The mitotic indices of the follicular cells (MIFC), the interfollicular cells (MIIFC), and cells belonging to follicles of different classes (MI_1, MI_2, etc., depending on their size) were determined as described previously [1-3]. The number of mitoses found in the peripheral zone of the gland, equal in thickness to the diameter of the field of vision of the microscope (7 × 40), and in the central zone, occupying the remainder of the section through the organ, was expressed as a percentage of the total number of mitoses. MI of the connective-tissue cells of the gland was calculated on the basis of the total number of thyrocytes examined. MI in the parathyroid epithelium was calculated for 10,000-14,000 cells. All MI values were expressed per 1000. The number of interfollicular cells also was determined as a percentage, the relative percentages of the numbers of different classes of follicles (folliculogram) were calculated, and the number of cells in follicles of each class (cytogram) was obtained as described previously [2]. The height of the cells in 100 follicles of the thyroid gland was measured in microns by means of a screw-adjusted ocular micrometer.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that follicles of class 2 accounted for more than half, and follicles of class 1 (microfollicles) for more than one-third of all follicles in the thyroid gland of the young rats. The folliculogram reflects the microfollicular type of gland structure observed under the microscope. The class 2 follicles also contained the largest cell population (Table 1). The size of the cell populations of the class 1 and 3 follicles was about the same, but it was 3.7-5 times smaller than that of the class 2 follicles. No change during the 24-h period was found in the relative percentages of follicles of different classes or in the number of cells in them, and there was likewise no change in the relative percentage of interfollicular cells in the gland. The absence of diurnal fluctuations in these values is evidently a feature of young rats, for in the gland of adult rats these parameters vary in the course of the 24 hours [2].

The follicular cells were highest at 1 a.m. (Table 2). Hypertrophy of the follicular epithelium may be evidence of increased excretion of hormonal products of the gland into the blood stream at this time, and consequently, of the existence of a diurnal rhythm of functional activity of the thyroid gland of the young rats.

The mitotic activity of the thyrocytes of young animals varies during the 24-h period (Fig. 1). The highest values of all mitotic indices studied were observed between 7 p.m. and 4 a.m. ($P = 0.012$ and $P = 0.050$ for the comparison with 7 and 10 a.m.). TMI changed in a similar manner to MIFC in the course of the 24 hours. The marked synchronization of the diurnal changes in MIIFC, MI_1, MI_2, and MI_3 must be emphasized. In the epithelium of the class 4 and 5 follicles the number of mitoses was very small so that no general principles governing diurnal fluctuations in the number of mitoses and in their cell populations could be established. Unlike the biphasic diurnal rhythm of mitosis in the thyroid epithelium of adult rats [2, 3], this rhythm in the young animals was thus monophasic. Another feature distinguishing the diurnal rhythm of reproduction of the thyrocytes in young rats was the similarity between the dynamics of the rhythms of mitosis in the follicles of different classes, whereas in the thyroid gland of adult animals the character of the rhythm

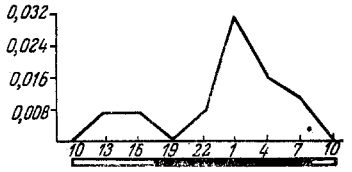

Fig. 2

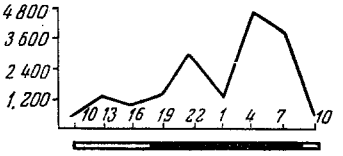

Fig. 3

Fig. 2. Changes in mitotic index in thyroid stroma of young rats during the 24-h period.

Fig. 3. Changes in mitotic index in parathyroid epithelium of young rats during the 24-h period.

of mitosis differs in the cell populations of different follicles [2, 3]. Consequently, in young animals the influence of development and differentiation of the thyrocytes on the diurnal changes in their mitotic activity has not yet become clearly manifested.

Determination of the mean values of the mitotic indices for the 24-h period in the thyroid epithelium of the young rats gave the following results: TMI 0.78, MIFC 0.73, MIIFC 1.49, MI_1 0.73, MI_2 0.86, MI_3 0.73, MI_4 0.10, and MI_5 0.03 (all values expressed in ‰). Clearly MIIFC was twice MIFC, but these differences are not significant. The first three classes of follicles likewise did not differ in their number of mitoses. However, mitotic activity fell sharply (by 7.6-26.9 times; $P = 0.001-0.021$) in the follicles of classes 4 and 5. Although differentiation of the thyrocytes in the young rats had no marked effect on the character of the diurnal rhythm of mitosis, the ability of the cells to divide by mitosis, as in the adult animals, depends on it.

On the average for the 24 hours, 27 and 73% of mitoses, respectively, were located in the central and peripheral zones of the gland. Between 1 and 4 a.m. the number of mitoses in the central zone was greater than in the period from 7 a.m. to 10 p.m. (38-44 and 17-28%, respectively; $P = 0.023$). Allowing for the fact that the area of the peripheral zone as a whole was smaller than the area of the central zone, these results point to a much higher intensity of cell division at the periphery of the gland than its central zone.

As Fig. 2 shows, between 7 a.m. and 10 p.m. the mitotic activity in the connective-tissue stroma of the thyroid gland was low. Mitoses were more numerous in this region in the period from 1 to 4 a.m. ($P = 0.013-0.026$). The diurnal rhythm of mitosis in the thyroid stroma of the young rats was thus monophasic and its dynamics was similar to that in adult rats [3, 4].

MI in the epithelium of the parathyroid gland of the young rats increased twice during the 24-h period: at 10 p.m. and at 4-7 a.m. ($P = 0.0001-0.040$ compared with the other times of day; Fig. 3). The mean diurnal MI was 2.40 ‰. By contrast to the biphasic rhythm of mitotic activity of the parathyroid cells in the young rats described above, a monophasic diurnal rhythm of mitosis is observed in the parathyroid gland of adult rats [3, 4].

The results of this investigation thus provide evidence of age differences in the diurnal rhythm of mitosis in the thyroid and parathyroid glands of rats, a decrease in mitotic activity in the course of differentiation of the thyrocytes, and the existence of organ and tissue specificity in the phase characteristics of the diurnal rhythm of cell reproduction.

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