EFFECT OF THYROXINE ON DIURNAL RHYTHMS OF MITOTIC ACTIVITY AND DURATION OF MITOSIS IN THE ESOPHAGEAL EPITHELIUM

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The diurnal rhythm of mitosis in the esophageal epithelium of rats after administration of thyroxine for 7 days is not synchronized with that of control animals. In both the control and experimental series the duration of mitosis in a diurnal rhythm is minimal at the height of mitotic activity. Consequently, stimulation of mitotic activity in the rhythm of both groups of animals is not connected with an increase in the duration of mitosis but with an increase in the number of cells taking part in mitosis per unit time.

Investigations [2, 3, 5, 7, 8] have established a decrease in the duration of mitosis at the height of mitotic activity during the 24-h period (in the epithelium of the small intestine, cornea, tongue, the epidermis of the skin, bone marrow and kidney cells). Since the duration of mitosis during the 24-h period is reflected in the values of the mitotic index, the possibility of changes in the rate of mitosis must be borne in mind when the action of hormones on mitotic activity is studied.

The object of the investigation described below was to study diurnal rhythms in the number of mitoses and in the duration of mitosis in the esophageal epithelium of rats receiving and not receiving thyroxine.

EXPERIMENTAL METHOD

Altogether 120 male albino rats weighing 50-60 g were used in the experiments. In the experiments of series I (80 rats) half of the animals received an intraperitoneal injection of the sodium salt of L-thyroxine in a dose of 10 μg/100 g body weight daily for 7 days at 8 a.m. A special experiment showed that the level of protein-bound iodine in the blood serum of the rats 5 and 10 days after injection of thyroxine in this dose averaged 13.5 and 14.0 μg% respectively, several times higher than the normal values.* The other half of the rats received the solvent of thyroxine (0.1 ml of 0.5×10^{-5} N KOH solution) in an equivalent volume at the same time. The animals of this series were sacrificed on the 8th day after injection of thyroxine, every 3 h starting at 10 a.m. and ending at 7 a.m. next day. In the experiments of series II (40 rats) the animals received thyroxine or its solvent as in series I but, in addition, they received an intraperitoneal injection of demecolcine in a dose of 5 mg/kg body weight at 10 a.m., 4 and 10 p.m., and 4 a.m. The rats were sacrificed 6 h after the injection of demecolcine, at 4 and 10 p.m. and 4 and 10 a.m. The overall mitotic index (OMI) in the esophageal epithelium, and the mitotic index in the stratum basale (MI_1) and stratum spinosum (MI_2) in promille were calculated for 20,000-30,000 cells. In series II, the corresponding indices after administration of demecolcine (OMI_{col}, MI_{1col}, and MI_{2col}) also were calculated. The duration of mitosis was determined by the formula $t_M = MI \times A/MI_{col}$, where A is the duration of action of the demecolcine.

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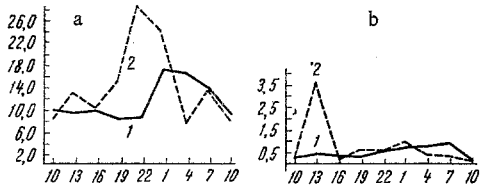

Fig. 1. Changes in mitotic activity in esophageal epithelium of control rats (1) and rats receiving thyroxine (2) during the 24-h period. Abscissa, time of day; ordinate: a) MI_1 (in $^0/_{00}$); b) MI_2 (in $^0/_{00}$).

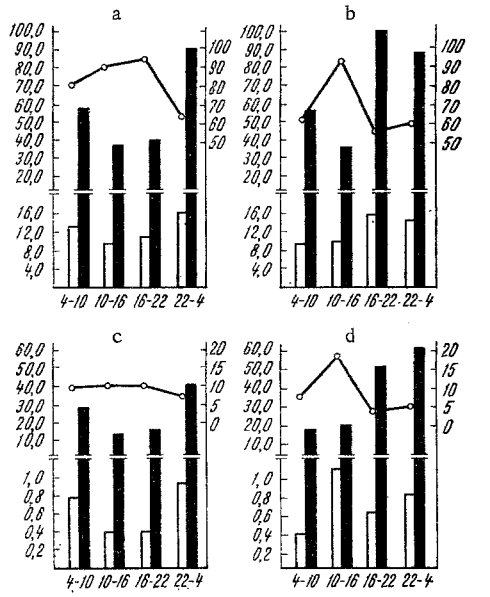

Fig. 2. Changes in duration of mitosis (curve), MI (unshaded columns), and MI_{col} (shaded columns) during the 24-h period in esophageal epithelium of control rats and rats receiving thyroxine. a and c) Stratum basale and stratum spinosum respectively of control rats; b and d) stratum basale and stratum spinosum respectively of rats receiving thyroxine. Abscissa, time of day; ordinate: left – MI (in $^0/_{00}$), right – t_M (in min).

EXPERIMENTAL RESULTS

The results of the experiments of series I show that a diurnal rhythm of mitosis exists in the stratum basale of the esophageal epithelium of intact rats, with a maximum between 1 and 7 a.m. and a minimum between 10 a.m. and 10 p.m. (P = 0.02-0.05; Fig. 1a). No significant changes in MI_2 were found during the 24-h period. The diurnal changes in OMI were analogous to those in MI_1. The mean daily values of MI_1 and MI_2 were 12.1 and 0.60 $^0/_{00}$, respectively. The results are in agreement with those described by other workers [1, 4, 5, 9] who found an increase in mitotic activity in the esophageal epithelium at night and in the morning. In animals receiving thyroxine an increase in MI_1 and OMI was observed between 10 p.m. and 1 a.m., i.e., 3 h earlier than in the control, and at this time the values of MI_1 and OMI were higher than those characteristic of other times (P = 0.001-0.050; see Fig. 1a). In addition, under the influence of thyroxine, OMI, MI_1, and MI_2 were increased at 1 pm., but whereas the changes in the first two indices were not significant, the increase in MI_2 was significant (P = 0.016; Fig. 1b). The mean daily values of MI_1 and MI_2 in animals receiving the hormone did not differ significantly from those in the control (14.5 and 1.0 $^0/_{00}$, respectively).

The results of the experiments of series II showed that higher values of MI_1 and MI_{1col} were observed in the control animals between 10 p.m. and 10 a.m. than between 10 a.m. and 10 p.m. (P = 0.001-0.050), with a maximum between 10 p.m. and 4 a.m. (Fig. 2a). Similar changes took place in OMI and OMI_{col}. Although MI_2 did not vary significantly during the 24-h period (as in the animals of series I), nevertheless, the values of MI_{2col} were higher between 10 p.m. and 10 a.m. than between 10 a.m. and 10 p.m. (P = 0.001-0.007; Fig. 2c), with a maximum at the same times as MI_{1col} (10 p.m.-4 a.m.). Consequently, the results for the accumulation of C-mitoses demonstrate a diurnal rhythm of mitotic division in the prickle cells similar to the rhythm in the number of mitoses in the basal cells. However, in this case the possibility of the more frequent migration of C-mitoses into the stratum spinosum from the stratum basale at a time when mitotic activity in the latter is increased must be borne in mind. The mean daily values of MI_1 and MI_2 differed by more than 20 times, whereas the mean daily values of MI_{1col} and MI_{2col} differed by only 2.5 times (57.8 and 23.6 $^0/_{00}$, respectively). These comparisons suggest that a certain proportion of the C-mitoses observed in the stratum spinosum are in fact C-mitoses which have migrated from the stratum basale. During the action of thyroxine, MI_1 was higher between 4 p.m. and 4 a.m. than between 4 a.m. and 4 p.m. (P = 0.010-0.050), and the highest value of MI_{1col} was observed between 4 p.m. and 4 a.m. (P = 0.001-0.010; Fig. 2b). Between 4 and 10 p.m., MI_{1col} for the animals receiving thyroxine was higher than for the controls (P = 0.001). Changes in OMI and OMI_{col} during the action of thyroxine were similar to the changes in MI_1 and MI_{1col}. Under the influence of the hormone no significant change in MI_2 took place during the 24-h period, but MI_{2col} was higher in the animals receiving thyroxine between 4 p.m. and 4 a.m. than at other times of day or night (P = 0.001), and it was higher at this period than in the control (P = 0.001-0.030; Fig. 2d). At the same time, the mean daily values of MI_{1col} and MI_{2col} were not significantly different from those in the control animals, with values of 72.1 and 39.2 $^0/_{00}$, respectively.

TABLE 1. Results of Calculations of P_M

	P_M in %, from t_M		P_M in %, from C-mitoses	
	normal	thyroxine	normal	thyroxine
Total cell population	14.4	21.0	14.3	21.1
Stratum basale	20.4	29.0	23.1	29.0

The results of the experiments of series I and II show that the diurnal rhythm of mitotic division in the esophageal epithelium of rats receiving thyroxine is not synchronized with the mitotic rhythm in intact animals, and this is reflected in a shift of the maximum of MI to the left by 3 h. The increase in MI_1 and MI_2 under the influence of thyroxine observed at 1 p.m. does not correlate with the changes in MI_{1col} and MI_{2col}, which are low at this time. This suggests that these changes in MI_1 and MI_2 do not reflect an increase in the number of dividing cells.

Since after a single injection of thyroxine in a dose of 10 $\mu g/100$ g the serum protein-bound iodine level does not return to normal until after 48 h [6], and a high concentration of hormonal iodine remains in the blood stream for 24 h, it can be postulated that this manifestation of a response of cell division in the esophageal epithelium to thyroxine only at a certain time of day is connected with the existence of a diurnal rhythm of sensitivity of cells to the hormone.

It is interesting to note that no significant changes could be found in the number of esophageal epithelial cells proliferating in the course of the 24-h period under the influence of thyroxine.

In no case of the investigation of mitotic activity were differences found in the value of the prophase index after injection of demecolcine or in its absence.

As Fig. 2a shows, the lowest value of t_M (64 min) in the basal cells was observed from 10 p.m. to 4 a.m., i.e., in the period of maximal mitotic activity, while the highest value (97 min) was found between 4 and 10 p.m., shortly before the rise in mitotic activity. The differences in the duration of mitosis are statistically significant. The results indicate that a diurnal rhythm of mitosis exists in the basal cells of the esophageal epithelium and they agree with the results of the writers' earlier investigations [5, 7] and with observations of other workers [2, 3, 8] to the effect that t_M is reduced at the time of the 24-h period when MI is high. They indicate, too, that the diurnal increase in mitotic activity is connected with an increase in the number of cells taking part in mitosis per unit time. In the prickle cells of the control animals there were no significant changes in t_M during the 24-h period (Fig. 2c). The mean daily changes in t_M in the general cell population of the esophageal epithelium and in the stratum basale and stratum spinosum were 62, 84, and 10 min respectively. The sharp increase in t_M in the stratum spinosum compared with the stratum basale evidently does not reflect the true relationships but is connected with an increase in the number of metaphases blocked by demecolcine in the stratum spinosum on account of their migration from the stratum basale, which takes place during the 6 h after injection of demecolcine into the animals.

In the experimental animals the greatest changes in t_M (98 min) in the stratum basale were observed between 10 a.m. and 4 p.m., when the number of mitoses was at a minimum. With an increase in MI_1 during the 24-h period (4-10 p.m.) t_M was reduced to 57 min ($P = 0.0001$; Fig. 2b). At this time, t_M for the experimental animals was about half its normal level. Changes in the diurnal dynamics of t_M under the influence of thyroxine are thus similar to the character of the changes in mitotic activity under these conditions, and the diurnal rhythms of t_M in control rats and rats receiving thyroxine, like the diurnal rhythms of mitotic activity, are not synchronized. In the prickle cells of the esophageal epithelium of the animals receiving thyroxine, t_M reached its highest value between 10 a.m. and 4 p.m. (20 min) and its lowest between 4 and 10 p.m. (4 min; Fig. 2d). The changes in t_M under the influence of thyroxine thus do not occur at all times, but only at certain times of the 24-h period. Both in the controls and in the experimental animals the amplitude of the variations in t_M was about the same. Consequently, under the influence of thyroxine t_M was not reduced to less than its minimal value during the natural diurnal rhythm. The results

of the experiments of series II showed that the increase in number of mitoses in the esophageal cells at 1 p.m. under the influence of thyroxine was due, not to a true increase in mitotic activity, but to the increase in t_M at that time. The mean daily values of t_M in the total esophageal epithelial cell population, and in the stratum basale and stratum spinosum after administration of thyroxine were 49, 70, and 9 min respectively, i.e., they were rather lower than in normal animals (by 16% in the stratum basale).

The results of the calculations of P_M (the number of cells dividing in the 24-h period) from t_M and from the number of C-mitoses are given in Table 1.

The time of renewal of the esophageal cells (T) was calculated from the formula

$$T = \frac{t_M \cdot N}{n},$$

where N is the total number of cells, and n the mean daily number of dividing cells (provided that P_c = 100%). The value of T under normal conditions for the whole population is 7.5 days, under the influence of thyroxine it is reduced to 5 days, and the corresponding values of T for the cells of the stratum basale are 4.8 and 3.3 days.

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