ACTION OF THYROXINE ON MITOTIC CYCLE OF CELL CULTURES

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Thyroxine, in a dose of 10 μg/ml, differed in its effect on the various periods of the mitotic cycle of HeLa cell cultures in the logarithmic and stationary phases of growth. In the logarithmic phase thyroxine shortened only the G_{2min}-period (by 2 h) and had no effect on the duration of the other phases of the mitotic cycle. In a stationary culture thyroxine shortened the total duration of the mitotic cycle by 3-7 h, mainly on account of a decrease in the time spent in the G_1 + ½M periods (by 4-8 h) and, to some extent, the G_{2min}-period (by 1 h). It is concluded that the hormone stimulates the entry of the cells into the mitotic cycle from the G_0-period in the stationary culture.

KEY WORDS: mitotic cycle; HeLa cell culture; thyroxine.

Thyroxine has been shown to influence cell division in vitro [1,2,4,6,11]. The effect of the hormone, just as in vivo, depends on the dose used. The writers showed previously that thyroxine, in a dose of 10 μg/ml, increases mitotic activity and the number of DNA-synthesizing cells in cultures of HeLa cells [3]. However, the mechanism of action of thyroid hormones on the mototic cycle has been inadequately studied in cell cultures. Data on the sensitivity of the individual phases of the mitotic cycle to thyroxine given in the literature are contradictory [2,4,6].

The object of this investigation was to study the action of thyroxine on the mitotic cycle of a HeLa culture in different stages of growth.

EXPERIMENTAL METHOD

A monolayer culture of HeLa S-3 cells was grown at 37°C in medium containing 45% Eagle's medium, 45% medium No. 199, and 10% bovine serum. For the experiments the cells were placed in penicillin flasks with coverslips; the initial density was 5×10^4 cells/ml medium. The sodium salt of L-thyroxine (Reanal) was used in a concentration of 10 μg/ml, dissolved in 0.1 N KOH. The hormone was added to the nutrient medium 24 h before the experiment began.

In the experiments of series I the culture of HeLa S-3 cells was used 36 h after subculture (logarithmic phase), and in series II 96 h after subculture (stationary phase).

The duration of the mototic cycle and its periods was determined from the change in the percentage of labeled mitoses [10] after incubation of the cells for 30 min with thymidine-^{3}H (specific activity 4.1 Ci/mmole). The cell culture was washed to remove the isotope in a solution of unlabeled thymidine (30 μg/ml) and cultivation continued in medium containing unlabeled thymidine (5 μg/ml). The cells were fixed for 36 h starting at the following times after addition of thymidine-^{3}H; 1,2,3, and 6 h and thereafter every 3 h. A mixture of ethanol and acetic acid (3:1) was used for fixation. Autoradiographs were prepared by the usual method, using type M (NIIKhimfoto) liquid photographic emulsion. The exposure was 2 days. A dividing cell was taken to be labeled if three or more grains of reduced silver were found above it. The preparations were stained with Mayer's hematoxylin.

To determine the percentage of labeled mitoses, from 2000 to 4000 cells were counted in four or five

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parallel samples obtained at each time of the investigation.

EXPERIMENTAL RESULTS

Curves of labeled mitoses for the normal and thyroxine-treated cultures in the logarithmic phase (2nd-3rd days of growth) are given in Fig. 1A. In the normal culture the duration of the mitotic cycle and of its individual periods was as follows: G_{2min} = 3 h, $G_2 + \frac{1}{2}M$ = 6 h, S = 9 h, $G_1 + \frac{1}{2}M$ = 6 h, T = 21 h; in the thyroxine-treated culture G_{2min} = 1 h, $G_2 + \frac{1}{2}M$ = 6 h, S = 9 h, $G_1 + \frac{1}{2}M$ = 6 h, and T = 21 h.

The absence of 100% of labeled mitoses on the curves can be explained on the grounds that addition of the isotope coincided with the passive phase of the rhythm of DNA-synthesizing cells (rhythmic changes were found in the number of DNA-synthesizing cells and of dividing cells in the HeLa culture). The results for the duration of the individual periods of the mototic cycle in the logarithmic phase of growth of the normal HeLa S-3 culture agree with those obtained by other workers [7,9,12] but do not confirm the results of Painter and Drew [8]. Treatment of the culture with thyroxine had no effect on the duration of the complete mitotic cycle, of the $G_1 + \frac{1}{2}M$, S, and $G_2 + \frac{1}{2}M$ periods. However, the duration of the G_{2min} period was reduced by two-thirds.

Curves of labeled mitoses in the normal and thyroxine-treated HeLa S-3 cultures in the stationary phase of growth (5th-6th days) are given in Fig. 1B. In the normal culture the duration of the mitotic cycle and of its individual periods was: G_{2min} = 2 h, $G_2 + \frac{1}{2}M$ = 4 h, S = 11 h, $G_1 + \frac{1}{2}M$ = 12-16 h, and T = 27-31 h; in the thyroxine-treated culture G_{2min} = 1 h, $G_2 + \frac{1}{2}M$ = 4 h, S = 12 h, $G_1 + \frac{1}{2}M$ = 8 h. and T = 24 h.

In the stationary phase of growth of the HeLa cell culture the duration of the mitotic cycle was thus longer than in the stationary phase, mainly on account of the longer time spent by the cells in the G_1 period and also, to some extent, in the S-period. This could also indicate the departure of some of the cell population from the

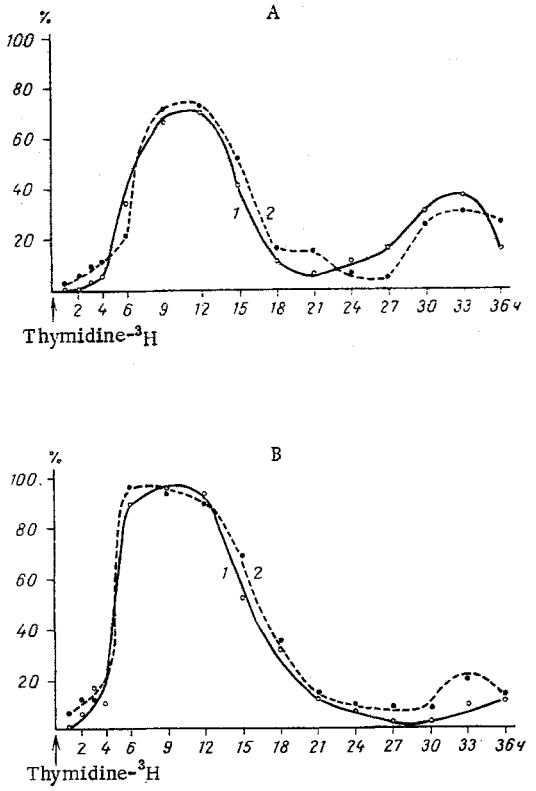

Fig. 1. Changes in number of labeled mitoses in normal and thyroxine-treated HeLa culture in logarithmic (A) and stationary (B) phases of growth: 1) normal cell culture, 2) culture treated with thyroxine. Ordinate, number of labeled mitoses (%); abscissa, time after injection of thymidine-^{3}H (in h).

mitotic cycle into the G_0 period [5]. Treatment of the cultures with thyroxine in the stationary phase of growth caused no significant changes in the duration of the S period and of $G_2 + \frac{1}{2}M$. However, unlike in the logarithmic phase the duration of the total mitotic cycle and of $G_1 + \frac{1}{2}M$ was reduced by 17 and 42% respectively. Furthermore, just as in the logarithmic phase, the duration of the G_{2min} period was reduced (by half).

Determination of the radioactive index 1 h after addition of thymidine-^3H revealed an increase in the number of DNA-synthesizing cells in both the logarithmic and the stationary phase by 30 and 20% respectively under the influence of the hormone. Consequently, thyroxine stimulates the entry of the cells into the mitotic cycle, and it takes place without any change in the duration of the S period of the mototic cycle. An increase in the number of DNA-synthesizing cells under the influence of thyroxine has also been found in a heteroploid culture of human renal epithelium [11]. In addition, shortening of the total mitotic cycle and of the S and, in particular, of the G_1 period has been demonstrated (on the 4th-5th day of growth) in the same culture [6]. The action of thyroxine on a culture of ovarian carcinoma cells in the stationary phase of growth was to shorten the mitotic cycle and the period of DNA synthesis [4]. The author cited considers that thyroxine induces the entry of cells into the mitotic cycle from the phase of the G_0 period. In stationary cultures of L and CaOv cells thyroxine induced the entry of the cells into the mitotic cycle from the resting periods (R_1 and R_2). The duration of the mototic cycle was reduced on account of the shorter time required for the cells to pass through all its periods [2].

The results of the present experiments are evidence that thyroxine stimulates the entry of the cells into the mitotic cycle from the G_0 period in stationary culture. At the same time, the duration of the G_{2min} period was shortened in both phases of growth of the culture. This was the result of the more rapid passage of the cells through the G_2 period. However, the possibility of entry of cells delayed in the G_2 period into the mitotic cycle under the influence of thyroxine cannot be ruled out for the stationary phase.

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