A THYMIDINE-H^3 STUDY OF THE MITOTIC CYCLE OF THE THYROID EPITHELIUM

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In recent years the mitotic cycle has been studied in various objects in vivo and in vitro by the method of autoradiography using thymidine-H^3 [5]. In this way the parameters of the mitotic cycle have been determined for several mammalian organs. As a rule, however, these investigations have been undertaken on organs characterized by high mitotic activity (skin, intestine, cornea, etc.) and by well marked physiological regeneration [2, 3, 6, 8, 10, 11, 13-15].

Meanwhile the parameters of the cell cycle have been inadequately studied in organs such as the thyroid, the liver, etc., possessing low proliferative activity and the presence of physiological regeneration in these organs in general is open to doubt [12].

The author submits that a definite relationship exists between the mitotic cycle and physiological regeneration, according to which organs capable of rapid regeneration possess a short mitotic cycle (intestine, cornea). For this reason, the determination of the parameters of the mitotic cycle in the thyroid may be expected to shed light on the problem of the presence or absence of physiological regeneration in that organ.

In the present investigation the mitotic cycle in the epithelium of the thyroid gland was studied by means thymidine-H^3, using the autoradiographic method.

EXPERIMENTAL METHOD

Experiments were carried out on male mice, C57B1×CBA hybrids weighing 19-20 g. Thymidine-H^3 with a specific activity of 2.5 Ci/mM was used as the DNA precursor. A single intraperitoneal injection of thymidine-H^3 was given in a dose of 0.8 μCi/g body weight in a volume of 0.1 ml of physiological saline. The animals were sacrificed by decapitation. Material was taken 1, 2, 3, 4, 5, 6, 8, 9, 10, 13, 14, 16, and 19 h after the injection of thymidine-H^3. The thyroid gland was fixed in Bouin's fluid and embedded in paraffin wax, and sections cut from it to a thickness of 5 μ were coated with type M (Motion Picture Research Institute—NIKFI) liquid emulsion; the exposure was thirty days. The sections were stained with Mayer's hematoxylin. On the average 50 mitoses were examined at each time, necessitating inspecting on the average 100,000 thyroid cells in each case.

EXPERIMENTAL RESULTS

By counting the numbers of labeled and unlabeled mitoses, the duration of the phases of the mitotic cycle could be determined (see figure).

As the figure shows, the first labeled mitoses were found 3 h after injection of the thymidine-H^3. They consisted of prophases with a few metaphases. The minimal duration of the G_2 period in the thyroid epithelium was therefore about 3 h.

The number of labeled mitoses 4 h after injection of thymidine-H^3 was 54.2%. All the prophases, and some of the metaphases and anaphases were labeled. At this time no labeled telophases were found among the 9 telophases seen at this time.

The number of labeled mitoses 5 h after injection of the thymidine-H^3 had risen to 77.8%. All the prophases, three-quarters of the metaphases, and half the anaphases were labeled, and the first labeled

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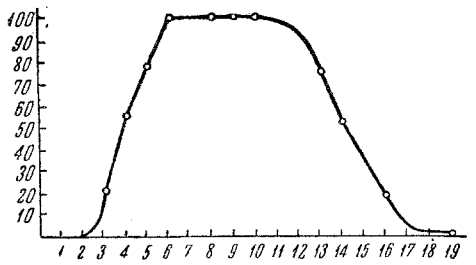

Changes in the percentage of labeled mitoses in the thyroid gland of immature mice. Abscissa) time after injection of thymidine-H^3 (in h), ordinate) number of labeled mitoses (in %).

telophases also were seen. This shows that the duration of mitosis in the thyroid epithelium was about 2 h.

All the mitoses were labeled 6 h after the injection of thymidine-H^3, so that the curve at this period was a plateau. All the mitoses were still labeled 8, 9, and 10 h after injection of the thymidine-H^3, but 13 h after injection the number of labeled mitoses fell to 75%, on account of the prophases.

The proportion of labeled mitoses fell to 52.6% 14 h after injection, to 19% 16 h after, and by 19 h after injection of the isotope no labeled mitoses were seen.

The results plotted on the graph thus showed that the mean duration of the period of synthesis in these experiments was about 10 h.

Since no second peak of labeled mitoses was observed, an attempt was made to determine the total duration of the mitotic cycle (T) by calculation, using the formula:

$$T = \frac{t_s}{I},$$

where t_S is the mean duration of the period S and I is the labeling index. In the present experiments the labeling index was determined in 10 mice and its value was 0.0035. By substituting the experimental values in the formula, it was found that the duration of the mitotic cycle in the thyroid epithelium was, on the average, 2857 h or 119 days.

The duration of mitosis was also determined by calculation [7] using the formula:

$$\frac{t}{T} = \frac{n}{N}.$$

where t is the duration of mitosis, n the number of mitoses, and N the number of undivided cells.

Since the ratio n/N varies during the twenty-four hours, the mean twenty-four-hourly value of the mitotic index obtained by author [4] in mice of the same line and weight was used in the calculation. The twenty-four-hourly value of n/N in these experiments was 0.00062. Hence the duration of mitosis was 1.7 h, a result close to that observed in the present experiments when the duration of mitosis was determined from the increase in the number of labeled mitoses phase by phase.

The following parameters were thus obtained for the mitotic cycle in these experiments: G_2 = 3 h, the duration of mitosis was about 2 h, S = 10 h, G_1 = 2842 h or 118.4 days, and the total duration of the mitotic cycle was 2857 h or 119 days.

No attempt was made to determine the parameters of the mitotic cycle in the parathyroid gland, although the first labeled mitoses in the parathyroid epithelium were observed incidentally 2 h after the injection of thymidine-H^3. These were labeled prophases (2 of 5) and metaphases (2 of 6). In the parathyroid epithelium the G_2 phase thus was shorter than in the thyroid epithelium, with a value of 2 h.

The results showed that the mitotic cycle of the thyroid epithelium is of long duration, as a result of the length of the G_1 phase. A similar picture has been observed [15] in the epidermis of the mouse's ear. Meanwhile the other phases of the mitotic cycle were similar in duration to those observed in other organs with high mitotic activity and a relatively short mitotic cycle. Nevertheless, these findings cannot be regarded as final, for they are only mean values, and more accurate measurements will have to be made in the future. No account was taken in the experiment of the twenty-four-hourly rhythm of DNA synthesis and of the proliferative pool (these have not yet been investigated for the thyroid).

The results obtained applied to the whole cell population of the thyroid epithelium. The parameters of the mitotic cycle in the follicular and interfollicular epithelium could not be differentiated because of the

extremely small number of mitoses found in the interfollicular epithelium. It is possible that they could be distinguished by their rhythms of DNA synthesis, by their proliferative pool, and by the parameters of their mitotic cycle, just as the different zones of the adrenal cortex possess their own distinct rhythms of twenty-four-hourly periodicity of mitosis [1].

Administration of thymidine-H^3 to the mice caused changes in the twenty-four-hourly periodicity of the mitoses in the thyroid epithelium. It was shown previously [4], that two peaks of mitotic activity occur in the thyroid gland of mice, one (the highest, 1.52%) at 13 h and the other (0.63%) at 1 h. A decrease in mitotic activity was observed in the thyroid in the evening (7 and 10 p.m.) and early morning (4 a.m.).

In the present investigation a higher level of mitotic activity was observed during the evening and night—from 8 p.m. until 6 a.m., i.e., in the first 2-10 h after the injection of thymidine-H^3. On the other hand, the mitotic activity of the thyroid epithelium in the late morning and afternoon was only a fraction of that observed in the earlier investigation, and it also was below the level of mitotic activity discovered in the evening and night in the previous investigation. A possible explanation of this is that thymidine-H^3 may be capable of modifying twenty-four-hourly rhythm of mitotic activity in the thyroid in a definite way.

The duration of the mitotic cycle in the thyroid epithelium is thus commensurate with the life span of mice. This may be evidence in support of the possibility of regeneration in the thyroid gland.

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