DNA SYNTHESIS IN FUNDAL CELLS OF THE RAT STOMACH IN HYPER- AND HYPOTHYROID STATES

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Large doses of thyroid extract (up to 100 mg/100 g body weight daily) increased DNA synthesis in the epithelium of the fundal glands of the rat stomach after four weeks and decreased it after 16 weeks. Small doses of thyroid extract (up to 25 mg/100 g body weight daily) increased DNA synthesis after both 4 and 16 weeks. The amplitude of the fluctuations in the diurnal curve of DNA synthesis was reduced four weeks after administration of 6-methylthiouracil (20 mg/100 g body weight daily), with a tendency toward normalization after 16 weeks. Total thyroidectomy reduced the fluctuations of the diurnal curve of DNA synthesis after 4 and 16 weeks. In both the last series DNA synthesis was reduced.

The process of cell renewal in the gastric mucous membrane is of interest both to normal physiology and to pathophysiology, with particular reference to oncology.

Since the 1950s, in the course of many investigations, mitoses have been found in the cells of the fundal glands of the rat stomach [10, 15, 19] and incorporation of labeled thymidine by the cells has been demonstrated [12-14]. The work of Timashkevich [4-6] and of Clark and Baker [8] has demonstrated the diurnal periodicity of cell division in the fundal glands of the stomach. The role of the thyroid gland in cell division has been demonstrated in the epithelial cells of the cornea, skin, and intestine [1, 3, 7]. Morphological and functional changes in the rat stomach under the influence of the thyroid gland have been studied [2, 9, 17]. However, no quantitative analysis has been made of cell renewal in the fundal glands of the stomach.

The object of this investigation was to determine the effects of hyper-, hypo-, and athyroid states in rats on DNA synthesis in the various epithelial cells of the fundal glands of the stomach.

EXPERIMENTAL METHOD

The experiments of series I were carried out on 115 male Wistar rats weighing 150-170 g. The rats were divided into five equal groups with 23 in each group. The rats of group 1 (thyrotoxicosis) received thyroid extract with their food daily as follows: first week 10 mg/100 g body weight, second week 25 mg/100 g, third week 40 mg/100 g, and fourth week 60 mg/100 g (total dose for the four weeks 945 mg/100 g). The rats of group 2 received thyroid extract from the first to the fourth weeks in a dose of 15 mg/100 g body weight (total dose 420 mg/100 g). The rats of group 3 were intact animals (control). The rats of group 4 received 6-methylthiouracil (6-MTU) in a daily dose of 20 mg/100 g body weight with their food (total dose 560 mg/100 g). Total thyroidectomy was performed on the rats of group 5.

The experiments of series II were carried out in a similar way but the hormonal treatment lasted 16 weeks. In group 1 the dose of thyroid extract from the 5th to the 10th week was increased to 80 mg/100 g, and from the 11th week until the end of the experiment to 100 mg/100 g (total dose 8505 mg/100 g); in

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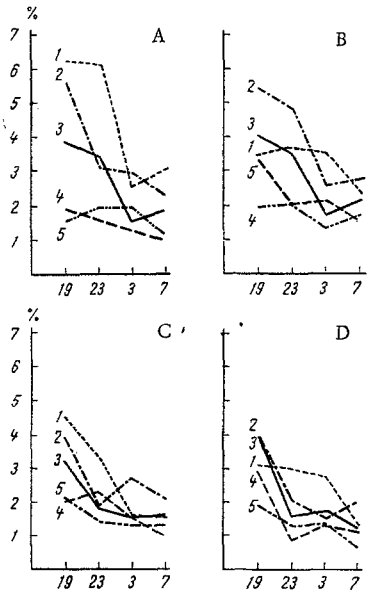

Fig. 1. Dynamics of DNA synthesis in mucous-forming cells in the fundal glands of the stomach: A) in surface epithelial cells after hormone treatment for 4 weeks; B) for 16 weeks; C) in mucous cells of the neck of the gland after 4 weeks; D) ditto after 16 weeks. Here and in Fig. 2: 1) thyroid extract in total dose of 945 mg/100 g (group 1); 2) thyroid extract in total dose of 420 mg/100 g (group 2); 3) control (group 3); 4) 6-MTU (group 4); 5) thyroidectomy (group 5). Abscissa, time of day when animals were sacrificed; ordinate, percentage of labeled nuclei for each type of cell in the fundal glands of the rat stomach.

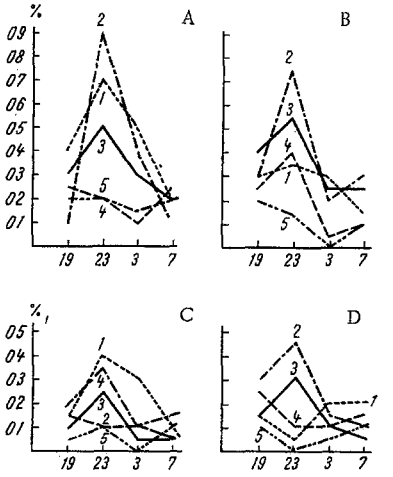

Fig. 2. Dynamics of DNA synthesis in specialized cells of the fundal glands of the stomach: A) in peptic cells after hormone treatment for 4 weeks; B) for 16 weeks; C) in oxyntic cells after treatment for 4 weeks; D) for 16 weeks.

group 2 from the 5th week and until the end of the experiment the animals received thyroid extract in a daily dose of 25 mg/100 g (total dose 2520 mg/100 g); the animals in groups 4 and 5 received the same treatment as in series I, but for a period of 16 weeks.

The animals were kept on the standard diet used at the Institute of Nutrition, Academy of Medical Sciences of the USSR; food was given to the rats daily between 10 and 11 A.M.

At the end of treatment with the hormones 5-6 rats from each group in each series were sacrificed at 7 and 11 P.M. and 3 and 7 A.M. Only water and no food was given to the animals for the 24 h before sacrifice. An intraperitoneal injection of 0.3 μCi thymidine-H^3 per gram body weight was given to each rat 1 h before sacrifice; the rats were killed by decapitation. The stomach was fixed with Carnoy's mixture and embedded in paraffin wax, and sections were cut to a thickness of 5-7 μ. The sections were coated with type M (NIKFI) photographic emulsion, exposed for 19 days, developed, and then stained with Meyer's hematoxylin and eosin. Labeled cells were counted in 50 longitudinally divided fundal glands of the stomach separately in cells of the surface epithelium, mucous cells of the neck of the gland, and oxyntic and peptic cells. The mean number of cells with labeled nuclei in 100 cells of each type was calculated. A cell was regarded as labeled if it was covered by five or more granules.

EXPERIMENTAL RESULTS

The mean number of cells in the fundal gland of the rat stomach was found to be 108, of which 41.7 are surface-epithelial, 15.1 mucous, 15.3 oxyntic, and 35.9 peptic. The distribution of labeled nuclei showed considerable inequality, as follows: on the average 43.7% of labeled nuclei were in surface-epithelial cells, 40.1% in mucous cells of the neck of the gland, 7.2% in oxyntic, and 9% in peptic cells.

The hormone treatment given caused significant changes in the process of DNA synthesis in all types of cells in the fundal glands of the rat stomach.

Curves showing the character of the changes in DNA synthesis are given in Figs. 1 and 2. It is interesting to note that significant differences were found during the period of maximal synthesis, which, as

the result showed, occurs between 7 and 11 P.M. (for the surface epithelia and mucous cells it was closer to 7 P.M. and for the oxyntic and peptic cells it was closer to 11 P.M.) and at other times they were virtually absent. Comparison of the curves obtained in the experiments of series I and II shows the role of the dose and duration of action of the thyroid hormone or 6-MTU in this process. During administration of thyroid extract in increasing doses an increase in DNA synthesis by all types of cells was observed after 4 weeks. For instance, at 11 P.M. 0.7% of the peptic cells (group 1) incorporated thymidine-H^3 compared with 0.5% of peptic cells in the control (group 3; Fig. 2A), while with a further increase in the dose and duration of administration there was a significant decrease in the intensity of DNA synthesis in the more specialized oxyntic and peptic cells (at 11 P.M. only 0.35% of the peptic cells incorporated thymidine-H^3 compared with 0.55% of peptic cells in the control; Fig. 2B) and to a lesser degree in the mucous-forming cells. Administration of small doses of thyroid extract (group 2) increased the intensity of DNA synthesis after both 4 and 16 weeks of the experiment. Hypothyroidism induced by administration of 6-MTU (group 4) like athyroidism after total thyroidectomy (group 5), led to a decrease in DNA synthesis and to a decrease in the amplitude of the diurnal fluctuations of its curve, thus, confirming the existing view that the thyroid gland plays a regulatory role in the diurnal periodicity of cell division [1].

This investigation thus showed that the thyroid gland regulates the process of renewal of the epithelium of the fundal glands of the stomach and that the regulatory effect is stronger on the more specialized epithelium. In addition, the important roles of the dose and time of administration of thyroid hormone are apparent. Failure to pay adequate regard to this fact, in the writer's opinion, is the commonest cause of the conflicting results obtained in different series of experiments to study the role of the thyroid gland in physiological processes.

LITERATURE CITED

1. I. A. Alov, Outlines of the Physiology of Mitotic Cell Division [in Russian], Moscow (1964), p. 42.
2. S. M. Lipovskii, Endocrine Glands and the Stomach [in Russian], Moscow (1969), p. 116.
3. Yu. A. Romanov, V. I. Prilutskii, S. M. Kremli, et al., in: Proceedings of the Third Conference on Problems of Regeneration and Cell Multiplication [in Russian], Moscow (1962), p. 134.
4. T. B. Timashkevich, Byull. Éksperim. Biol. i Med., No. 1, 100 (1963).
5. T. B. Timashkevich, Mitotic Behavior in the Mucous Membrane of the Rat Stomach. Author's Abstract of Candidate's Dissertation, Moscow (1967).
6. T. B. Timashkevich, in: Regeneration and Cell Division [in Russian], Moscow (1968), p. 415.
7. I. É. Épova, Byull. Éksperim. Biol. i Med., No. 2, 80 (1957).
8. R. H. Clark and B. L. Baker, Am. J. Physiol., 204, 1018 (1963).
9. G. R. Crean, Vitamins and Hormones, 21, 215 (1963).
10. T. Hunt, Anat. Rec., 112, No. 2, Suppl. 346 (1952).
11. T. Hunt, Anat. Rec., 127, 505 (1957).
12. T. Hunt and E. Hunt, Anat. Rec., 142, 505 (1962).
13. B. Messier, Anat. Rec., 136, 242 (1960).
14. B. Messier and C. Leblond, Am. J. Anat., 106, 247 (1960).
15. C. E. Stevens, Anat. Rec., 112, 392 (1952).
16. C. E. Stevens and C. Leblond, Anat. Rec., 115, 231 (1953).
17. D. C. H. Sun, H. Shay, H. Sipler, et al., Gastroenterology, 27, 189 (1954).