CHANGES IN THE SORPTION CAPACITY AND ISOELECTRIC POINT OF THYROID CELLS SUBJECTED TO THE ACTION OF 6-METHYLTHIOURACIL

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Intensification in the functional activity of the thyroid gland, and, in particular, its reaction to the stimulus of thyrotropic hormone (both endogenous and exogenous), is essentially manifested by an increase in the volume and height of the cells in the follicular epithelium [1, 2, 4], which occurs with such regularity that it is usually considered as the criterian of the functional state of this organ. The increase in the volume of activated thyroid cells is related to their resorption of the products of intrafollicular colloid proteolysis, which is an intermediate stage in the processes leading to the secretion of the thyroid hormones from the thyroid gland into the vascular bed, and comprises an essential link in the secretory cycle of this gland.

In order to clarify the nature of the factors that cause intensified absorption of the products of colloid splitting by the activated cells in the follicular epithelium, it was necessary to determine the change in their sorption capacity. Along with this, since intensified output of secretion in every gland (and in the thyroid, the processes of secretion of thyroid hormones into the blood and lymphatic circulations) is accompanied by a clear intensification of the dissimilatory metabolic phase (which is manifested by increased oxygen absorption and carbon dioxide excretion by the activated gland parenchyma) [3], the reaction of the thyroid gland to the stimulatory action of thyrotropic hormone should be accompanied by changes in the colloid composition of the thyroid cells, and, in particular, by a shift in the isoelectric point (IEP) of their cytoplasm and nucleus [10, 11].

A significant increase in the production and secretion of endogenous thyrotropic hormone may be caused by the action of thyrostatic substances [3, 9, 15]. These agents, directly effecting the thyroid gland, primarily depress the respiration of its parenchyma [8]; in turn, blockade of thyroid hormone formation causes an intensification of thyrotropic functioning on the part of the hypophysis, as a result of which a secondary action is exerted on the thyroid gland [4].

In order to resolve this problem of the dynamics behind the changes in sorption capacity of the thyroid parenchyma, and the shift in position of the IEP for the cytoplasm and nucleus of its cells, we studied the development of the thyroid gland's reaction to 6-methylthiouracil. On stopping the action of the thyrostatic agent, the thyroid gland returns to its original state relatively quickly (provided the action of 6-methylthiouracil is not too prolonged) [5, 6, 7]. Thus, it was also necessary to investigate the nature of the normalization in the changes in sorption capacity and in the IEP shift during the period of normalization of the thyroid gland.

EXPERIMENTAL METHOD

The experiments were carried out on male rats, weighing 180-200 grams, and male rabbits, weighing 1500-1800 grams. The animals received 6-methylthiouracil daily with their food, in a dosage of 10 mg per 100 grams of body weight. The thyroid glands were removed for investigation after 5, 10, 15, 20, 25, and 30 days of treatment with 6-methylthiouracil, and 1, 2, 3, 4, 5, 8, 10, and 15 days after we stopped administering this thyrostatic substance. The dynamics of the developing (or regressing) functional excitation of the thyroid gland were evaluated from the increase in its relative weight (during the action of 6-methylthiouracil) or from its decrease (in the period of restitution), and from the increase or decrease in the mean height of the follicular epithelial cells. The latter were measured in sections of the thyroid glands, fixed in Zenker's solution with formalin and stained according to the azan method. The results of the measurements were subjected to variation-statistics analysis.

TABLE 1. Alteration of the Biocolloids in the Thyroid Parenchyma of Rabbits Subjected to the Action of 6-Methylthiouracil, and During the Period of Restitution of the Gland

Time of expt. (in days)	Mean relative wt. of gland (in mg)	Mean height of follicular cells (in μ)	Sorption of neutral red (%)	Isoelectric point (expressed as pH)		
				of the follicular cells' cytoplasm	of the follicular cells' nucleoplasm	Control
	6,77	6,70±02	100,0	3,50	3,40	3,88
5	8,40	7,30±01	74,0	3,50	3,30	3,88
10	10,02	9,40±09	70,0	3,50	3,30	3,69
15	13,07	9,80±01	63,0	3,30	3,20	—
20	22,40	12,90±07	54,0	3,30	3,10	—
25	25,20	14,30±03	92,0	3,20	3,00	—
30	18,71	10,80±07	100,0	3,10	2,89	—
30/3	18,00	8,30±05	93,0	3,50	3,30	3,69
30/5	16,10	8,10±08	58,4	3,50	3,30	4,08
30/7	15,00	8,00±02	53,0	3,50	3,30	4,50
30/8	14,20	7,30±04	59,0	3,46	3,28	4,70
30/10	13,60	7,42±03	66,0	3,46	3,28	4,26
30/14	12,85	7,10±08	64,0	3,28	3,10	3,88
30/15	14,51	7,00±04	74,5	3,28	3,10	3,88

Note. In the far left column, in the numerator – duration of exposure to 6-methylthiouracil, in the denominator – duration after stopping the administration of the drug.

In the far right column, the hyphen designates that the colloid was completely discharged, and it was not possible to determine the position of its IEP.

TABLE 2. Alteration of the Biocolloids in the Thyroid Parenchyma of Rats Subjected to the Action of 6-Methylthiouracil, and During the Period of Restitution of the Gland

Time of expt. (in days)	Mean relative wt. of gland (in mg)	Mean height of follicular cells (in μ)	Sorption of neutral red (%)	Isoelectric point (expressed as pH)		
				of the follicular cells' cytoplasm	of the follicular cells' nucleoplasm	Control
	12,26	7,40±03	100,0	3,59	3,48	3,79
3	14,10	9,61±10	103,0	3,60	3,42	3,73
5	20,82	10,37±08	105,0	3,53	3,30	3,60
10	25,50	10,95±01	53,0	3,45	3,28	3,53
15	29,10	13,86±06	53,0	3,45	3,10	
20	36,56	14,81±11	83,0	3,25	3,00	»
25	42,16	15,46±02	72,5	3,10	2,85	»
30	50,60	14,12±07	68,3	3,10	2,85	»
30/1	39,30	13,98±03	69,4	3,45	2,93	3,50
30/2	38,00	12,30±06	71,3	3,27	3,00	3,18
30/3	30,18	11,92±01	80,0	3,45	3,10	3,27
30/4	28,10	10,68±04	77,0	3,20	3,18	3,35
30/5	26,60	10,68±08	76,0	3,20	3,18	3,40
30/8	22,00	9,23±03	80,2	3,27	3,18	3,27
30/10	28,38	8,89±08	104,0	3,54	3,42	4,32
30/15	17,08	7,75±09	138,0	3,75	3,68	4,32

Note. In the far left column, in the numerator – duration of exposure to 6-methylthiouracil, in the denominator – duration after stopping the administration of the drug; n/d – determination not performed.

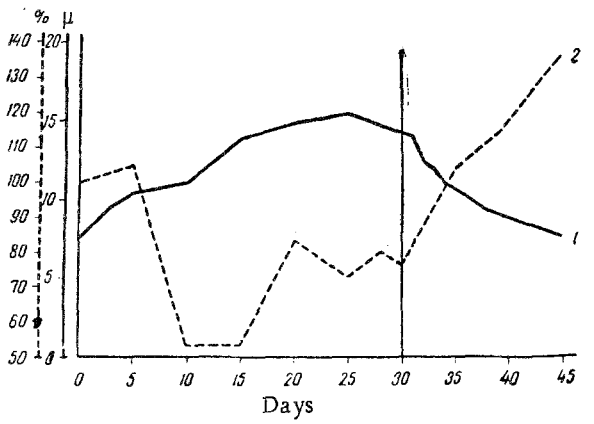

Fig. 1. Alteration in the mean height of the follicular epithelial cells (1), and in the neutral red sorption intensity of the follicular epithelium (2), in the thyroid glands of rats, during the period of 6-methylthiouracil administration, and in the period of restitution.

In each of the above-mentioned time periods, we determined the sorption capacity of the thyroid parenchyma and the change in the IEP position of its cells. These determinations were carried out on 6 thyroid glands. Change in the sorption capacity of the thyroid parenchyma was established by using the method of Nasonov and Aleksandrov [12, 13], judging from the intensity of absorption of neutral red. Change in the position of the IEP for the cytoplasm and nucleus of the thyroid cells, and also for the intrafollicular colloid, was established by applying the method of Pischinger and Roskin [14, 19], reading the color reactions of the sections of thyroid gland, fixed in 10% neutral formalin and stained in solutions of toluidine blue and acid fuchsin at different pH levels.

EXPERIMENTAL RESULTS

As can be seen from Tables 1 and 2, the reaction of the thyroid gland to the action of 6-methylthiouracil reached full development on the 25th-30th day, since by this time the increase in the mean relative weight of the gland, and the mean height of the cells in the follicular epithelium, reached its maximum level.

However, despite the progressive increase in volume of the follicular epithelial cells, the intensity of their absorption of neutral red during this period initially not only did not increase, but, on the contrary, weakened markedly. The intensity of absorption of the thyroid parenchyma began to increase somewhat only as the height of the cells approached the maximum enlargement (Fig. 1).

During the period of restitution, the sorption capacity of the thyroid parenchyma was also diminished, and its return to normal, or to even a certain intensification above normal, occurred considerably after the normalization of the height of the follicular epithelial cells, and thus, the decrease in their volume. A comparison of the data presented indicates that the increase in dimensions of the cells, regularly accompanying the increase in functional activity of the thyroid gland, is not determined by an intensification in the sorption capacity of the cytoplasm, but by other factors. The physiological significance of this deviation between the increase in volume of the follicular epithelial cells and the sorption intensity becomes understandable if one takes into consideration that the fluid products resulting from hydrolysis of the intrafollicular colliod, which are absorbed by the thyroid cells, are subject to rapid discharge from the latter into the blood and lymphatic circulation, which would be hindered by their strong adsorption onto the cytoplasmic micelles Assuming that activation of the thyroid gland is connected with an increase in oxygen absorption by its parenchyma, and thus, with a clear intensification of dissimilatory processes [2], it may be postulated that the forced entrance of the fluid products of intrafollicular colloid proteolysis into the cells of the thyroid epithelium is caused, to a large degree, by an elevation in the intracellular osmotic pressure.

With progressive excitation of the thyroid gland, the cytoplasmic IEP of the follicular epithelial cells gradually shifted toward the acid side (pH from 3.5-3.6 to 3.1) (Fig. 2). This shift developed relatively slowly and reached its most extreme level at the time of maximum enlargement of the mean thyroid cell height (see Tables 1 and 2). The shifts in IEP of the nucleus were somewhat more marked. Cessation of the action of 6-methylthiouracil was accompanied by a rapid reverse shift of the IEP position, both for the cytoplasm and the karyoplasm, toward the alkaline side.

Although here was a certain parallel between the degree of enlargement of the follicular epithelial cell dimensions and the shift in position of the cytoplasmic and karyoplasmic IEP, the connection between these phenomena is apparently not a direct one. Thus, after cessation of the action of 6-methylthiouracil, the IEP immediately shifted toward the alkaline side, but the decrease in height of the markedly swollen thyroid cells occurred relatively slowly, and lagged considerably behind the IEP changes. The position of the intrafollicular colloid IEP underwent the same clear changes in connection with the activation and restitution of the thyroid gland (see Fig. 2). The period of colloid splitting was characterized by a significant shift in the position of its IEP toward the acid side (pH from 3.88-3.79 to 3.69-3.53). Later, at the height of the functional excitation that developed, it was impossible to determine the IEP of the colloid, since the marked resorption of the products of its hydrolysis by the cells of the follicular epithelium, and the discharge of these resorbed substances into the vascular bed, led to almost complete evacuation of the colloid from the cavity of the follicles.

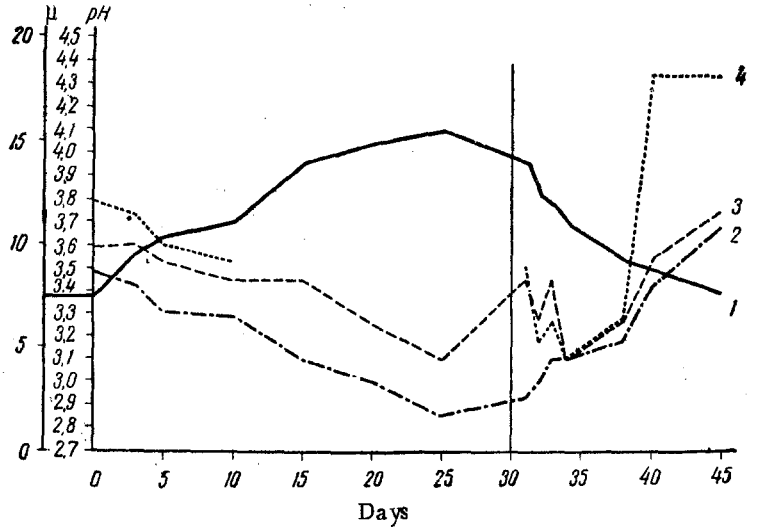

Fig. 2. Alteration in the sorption capacity and position of the IEP of the thyroid parenchyma in rats, during the period of 6-methylthiouracil administration and in the period of restitution. 1 – mean height of the follicular cells; 2 – IEP of the karyoplasm in the follicular cells; 3 – IEP of the cytoplasm in the follicular cells; 4 – IEP of the colloid in the follicular cells.

As the colloid collected, once again synthesized during the period of restitution, its IEP began to shift toward the alkaline side, not rectilinearly but rather with certain intermediate fluctuations toward the acid side. Shift of the colloidal IEP toward the acid side during activation of the thyroid gland is clearly related to its proteolytic splitting [16, 17, 18], since a significant reduction in the degree of shift toward the acid side is obviously one of the conditions for synthesis and accumulation of new colloid during the period of restitution.

SUMMARY

Increase of the thyroid functional activity caused by 6-methylthiouracyl and resitution of the gland after this thyreostatic agent is removed is accompanied by shifts of sorption ability and isoelectric point (IEP) position of thyroid cells. Increase of the average height of thyroid cells is accompanied by a decrease of their sorption capacity and acidotic derangement of shift of the karyoplasm, cytoplasm and thyroid colloid IEP position. After the threostatic agent is removed the sorption capacity restores to the initial value but the rate of restitution is less than that of decrease of thyroid cells height. Therefore it is reasonable to suppose that resorption of products of colloid hydrolysis influencing volume increase of thyroid cells during their activation is due to sorption, but to some other phenomena. Restitution is characterized by an alkalotic derangement of IEP position of cytoplasm, nuclei of follicular epithelium cells and thyroid colloid.

LITERATURE CITED

1. B. V. Aleshin, Probl. endokrinol., 1938, Vol. 3, No. 2, p. 5.
2. B. V. Aleshin and P. F. Sarenko, Uchen, zapiski Khar'kovsk. un-ta, 1947, Vol. 25, p. 203.
3. B. V. Aleshin and N. S. Demidenko, Arkh. anat., 1952, No. 3, p. 82.
4. B. V. Aleshin, and N. S. Demidenko, in the book: Neural Regulation of Endocrine Functions [in Russian]. Kiev, 1959, p. 140.
5. B. V. Aleshin and V. V. Mamina, Probl. endokrinol., 1961, No. 4, p. 3.
6. B. V. Aleshin, in the book: Advances in Thyroid Research. Oxford, 1961, p. 332.
7. B. V. Aleshin, et al., Activ. nerv. sup. (Praha), 1961, Vol. 3, p. 289.
8. V. M. Gordienko, in the book: Goiter Disease [in Russian]. Kiev, 1959, Vol. 2, p. 338.
9. N. S. Demidenko, Theses from the Reports of the 5th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian]. L., 1949, p. 243.
10. Yu. V. Krivobok, The Reaction of Thyroid Cells to the Action of Radioactive Iodine. Diss. kand. Kharkov, 1958.
11. Yu. V. Krivobok, in the book: Questions in Endocrinology [in Russian]. Kharkov, 1958, p. 45.
12. D. N. Nasonov and V. Ya. Aleksandrov, On the Reaction of Living Material to External Stimuli [in Russian]. M. – L., 1940.

13. D. N. Nasonov and I. P. Suzdal'skaya, Arkh. anat., 1953, No. 4, p. 32.
14. G. I. Roskin, Uspekhi sovr. biol., 1946, Vol. 22, No. 2, p. 247.
15. Yu. B. Skebel'skaya, Dokl. AN SSSR, 1950, Vol. 75, No. 3, p. 473.
16. E. DeRobertis, Anat. Rec., 1941, Vol. 80, p. 219.
17. E. DeRobertis and W. W. Nowinski, J. clin. Endocr., 1946, Vol. 6, p. 235.
18. E. DeRobertis, Ann. N. Y. Acad. Sci., 1949, Vol. 50, Art. 5, p. 317.
19. A. Pischinger, Z. Zellforsch., 1925-1926, Bd. 3, S. 169.

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