PARAFOLLICULAR CELLS IN THE REGENERATING THYROID GLAND

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KEY WORDS: thyroid gland; regeneration of thyroid parenchyma; parafollicular cells (calcitoninocytes).

The writers showed previously that the functional state of the parafollicular cells (calcitoninocytes) is closely dependent on nervous impulses reaching the thyroid gland from cervical sympathetic ganglia, and also that a parallel exists between the activity of these neuroendocrine cells and the degree of functional excitation of the thyrocytes [2, 3]. However, if this assumption is valid, activity of the calcitoninocytes should increase in the thyroid stump left behind after partial thyroidectomy, the functional activity of which increases as a compensatory reaction of the body to diminished thyroid hormone formation. The aim of the present investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Male rabbits weighing 2.5 kg were subjected to partial thyroidectomy (resection of the lower lobe of the thyroid gland, leaving the right lobe intact). Ten days after this operation the right (control) and left (regenerating) lobes of the thyroid gland were removed and treated by Sawicki's method [7]. The state of the calcitoninocytes was assessed from the degree of their swelling, with planimetric determination of the areas of cross section of these cells, projected on paper by means of a drawing apparatus.

EXPERIMENTAL RESULTS

In the lobe of the thyroid gland from which the lower half had been resected the compensatory increase in functional activity of the follicular epithelium was distinctly expressed, as shown by a marked increase in volume and height of the thyrocytes, intensive reabsorption of intraluminal colloid, and hyperemia of the parenchyma. However, despite this undoubted increase in thyrocyte activity, weakening of activity of the parafollicular cells was obvious. The number of calcitoninocytes was distinctly less than in the intact zone (Fig. 1), and the reduction in their size was particularly marked (Table 1). Whereas in the intact lobe of the thyroid gland their area of cross section was $210.5 \times 16\ \mu^2$, in the regenerating lobe it did not exceed $134.7 \times 1.2\ \mu^2$, i.e., it was only about 64% of the former.

However, as we showed previously [1], the mean area of cross section of calcitoninocytes in normal rabbits is $221.2 \pm 2.5\ \mu^2$. Thus in rabbits undergoing partial thyroidectomy, re-

TABLE 1. State of Parafollicular Cells in Regenerating Rabbit Thyroid Gland

Test object	Mean area of cross section of calcitoninocytes, μ^2
1. Intact rabbits	221,2±2,5
2. Lobe of thyroid gland left intact	210,5±1,6 $P_{2-1}<0{,}001$
3. Regenerating lobe (after resection of its lower half)	134,7±1,2 $P_{3-2}<0{,}001$

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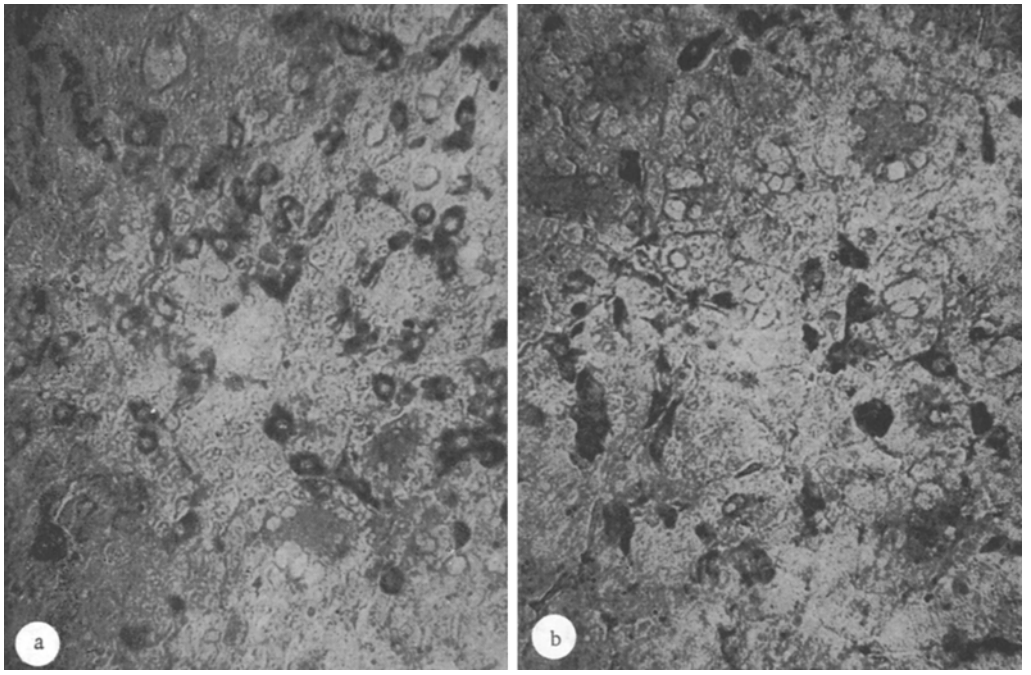

Fig. 1. Calcitoninocytes in thyroid parenchyma: a) in intact lobe of thyroid gland, b) in regenerating lobe. Stained by Sawicki's method, 240 ×.

duction of the parafollicular cells not only occurred in the regenerating lobe, but also spread to the lobe left intact (although in the latter the decrease did not exceed 5% of the normal values). This comparison shows that after partial thyroidectomy the secretory activity of the parafollicular cells becomes distinctly weaker, and it no longer follows a parallel course with the state of the thyrocytes.

Since no signs of atrophy or necrosis of calcitoninocytes were found in the regenerating throid parenchyma, the decrease in their number in this situation was due, it must be supposed, not to their destruction but to other causes. Reactions by which parafollicular cells are identified in histological preparations include argyrophilia (or osmiophilia) of their secretory granules, specific fluorescence of the granules in UV light after treatment of the sections with formaldehyde vapor and, finally, metachromatic staining of the granules with toluidine blue after preliminary hydrolysis. All the reactions listed above are linked with the presence of monoamines (dopamine or serotonin) in these granules. Consequently, these reactions in fact reveal, not calcitoninocytes as such, but only their secretory granules. That is why, if the number of these secretory granules in the cytoplasm is reduced, it is impossible to identify calcitoninocytes reliably in thyroid sections. For example, in thyrotoxicosis the number of parafollicular cells in the goitrous thyroid parenchyma increases, but they are empty, for the secretion which they synthesize is quickly released, is not retained in the cytoplasm, and consequently the calcitonins begin to exhibit their own specific reactions only after receiving an additional supply of dihydroxyphenylalanine (dopa) as a precursor of dopamine [4]. In the same way, parafollicular cells in the thyroid glands of bats, during hibernation, likewise cannot be detected in histological preparations, not because they are not present but because, under hibernation conditions, they cease to function and, consequently, the formation of secretory granules ceases [5, 6]. As regards conditions under which the calcitoninocytes find themselves in the regenerating thyroid gland, here it must be emphasized that under circumstances the concentration of acetylcholinesterase in the gland is increased, evidence of raised cholinergic tone. However, the data in the previous communication show that secretory activity of calcitoninocytes is stimulated by sympathetic impulses reaching the thyroid gland. Consequently, predominance of parasympathetic impulses received by the thyroid gland implies the reduction of sympathetic influences to which the thyroid parenchyma is exposed, with the result that the secretory activity of the parafollicular cells is depressed to such an extent that many of them cease to produce specific granules, and on that account they cannot give specific reactions in histological preparations.

Since secretory activity of the calcitoninocytes is reduced when parasympathetic influences predominate, despite an increase in functional activity of the thyrocytes (due, it must be supposed, to an increase in thyrotrophin secretion by the adenohypophysis, which is intensified after partial thyroidectomy), it follows from the above account that, first, the functional state of the parafollicular cells depends on direct nervous impulses to a much greater degree than thyrocytes and, second, functional shifts in the thyrocytes and calcitoninocytes begin to follow the same direction only when adrenergic influences in the thyroid gland predominate over cholinergic; third, and last, the cause of this parallel is not the influence of thyrocytes on calcitoninocytes or of calcitoninocytes on thyrocytes, but sympathetic impulses reaching the thyroid gland and acting simultaneously on both thyrocytes and calcitoninocytes.

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CYTOPHOTOMETRIC ANALYSIS OF TRANSITION FROM PHASE G_0 TO PHASE G_1 OF THE CELL CYCLE

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Recently in connection with the appearance of modern precision scanning cytophotometers equipped with computers, new prospects have opened for the automatic analysis of the image of a cell and its individual structures. In particular, investigation of the geometric parameters of cell nuclei stained by Feulgen's method and of the distribution of optical densities has made it possible to determine quantitative changes in the ratio between condensed and diffuse chromatin and the geometry of the cell nucleus in cells of eukaryotes during their stimulation [8, 10], virus infection [7, 9], in the course of acid hydrolysis of DNA [4], and after exposure to ionizing radiation [5].

The object of this investigation was to study the possibility of separating cells in phases G_0 and G_1 of the cell cycle on the basis of cytophotometry of cell nuclei stained by Feulgen's method.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes from a blood donor were isolated and cultured with PHA by the method described previously [2]. The cells were cultured for 24, 48, and 72 h. Films were fixed in a mixture of ethanol and acetone (1:1) for 30 min at room temperature. Hydrolysis in 5 M HCl was carried out at 37°C for 15 min, after which the preparations were stained in Schiff's reagent, made from basic fuchsine (from Reanal, Hungary) [3].

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