MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF REPAIR PROCESSES IN THE RESECTED RAT THYROID GLAND

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The main attention in the study of the dynamics of physiological and reparative regeneration of the mammalian thyroid gland (TG) has been concentrated on the morphology and function of the follicular cells [5, 12], which produce the thyroid hormone thyroxine (T_4) and tri-iodothyronine (T_3), and which are under the control of pituitary thyrotrophic function. It is also known that the parenchyma, the main part of the gland, contains not only thyrocytes (A and B cells), but also perifollicular or C cells. These cells possess structural and functional autonomy and are concerned with the production of several hormones: calcitonin (CT), which controls the blood calcium level, and serotonin and somatostatin [1, 2, 7, 9, 13]. The number and distribution of C cells are heterogeneous and depend on the age and species of the animals, and also on the character of the experimental procedures [10, 11]. They are regulated mainly by the blood Ca level [14]. It is considered that this is an independent cell system, in which the number of cells increases through self-reproduction by mitosis, and not by transformation from follicular thyrocytes [6].

The aim of this investigation was to study the dynamics of morphological and physiological parameters of the albino rat TG at various times after resection of two-thirds of the gland.

EXPERIMENTAL METHOD

Experiments were carried out on 190 noninbred male albino rats. The animals were killed in the morning under hexobarbital anesthesia, 3, 12, and 24 h and 2, 5, 7, 15, and 30 days after resection of TG. The control group consisted of intact animals. Material for study was treated by histologic and some histochemical methods. The C cell population was studied in sections impregnated by Gimelius' method in De Grandi's modification or stained by Sawicki's method [15]. Small fragments of TG for electron-microscopic study of thyrocytes and C cells were fixed in glutaraldehyde and OsO_4 and embedded in a mixture of Epon and Araldite M. Ultrathin sections were stained with uranyl acetate and examined in the IÉM-7A microscope. Repair processes were evaluated quantitatively by studying the trend of gravimetric parameters of the gland, the relative numbers of tissue components, and the height of the gland cells. Proliferative activity was estimated by counting mitotic activity of the thyrocytes (in 5000-6000 cells), and also by autoradiography with [^{3}H]thymidine.

In all series of experiments the basic parameters of the hormonal profile of TG were studied: intravital scanning with ^{131}I, determination of T_3, T_4, CT, endogenous thyrotrophic hormone (TTH), and parathyroid hormone (PTH) levels in the blood serum, using commercial radioimmunoassay kits. To evaluate the state of the C cell system, besides CT and its physiological antagonist PTH, the blood CA concentration was determined biochemically.

EXPERIMENTAL RESULTS

Resection of two-thirds of TG was followed in the initial postoperative period (3-24 h) by marked structural changes in all the residual part of the gland, as shown by complete or partial destruction of follicles ad-

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TABLE 1. Morphologic Parameters of the Regenerating Rat TG

Parameter studied	Control	Resection, days				
		1/2	2	5	15	30
Area of "new" tissue in zone of resection, percent	—	—	0,17±0,01	0,96±0,07	4,81±0,69	5,83±0,36
Height of thyrocytes, μ	4,5±0,12	4,6±0,31	6,5±0,21	6,8±0,33	5,4±0,26	5,2±0,22
P			<0,001	<0,001	<0,005	<0,025
MI of thyrocytes, percent	0,849±0,04	—	3,571±0,52	3,912±0,46	1,283±0,41	0,985±0,07
P			100,0	<0,001	>0,25	>0,25
Number of C cells (per field of vision)	2,0±0,3	1,44±0,18	1,93±0,23	2,188±0,29	3,277±0,31	1,64±0,24
P					<0,005	

TABLE 2. Parameters of TG Function in Rats at Different Times after Resection

Parameter of hormonal profile	Control	Time after resection						
		3 h	12 h	24 h	2 days	5 days	15 days	30 days
T_4, μg/100 ml	4,9±0,27	—	—	5,55±0,87	—	5,4±0,8	5,2±0,6	6,44±0,85
T_3, ng/ml	1,45±0,16	—	—	4,3±0,5	—	4,8±0,5	4,68±0,5	3,5±0,4
TTH, μg/liter	2,8±0,24	2,5±0,44	2,61±0,1	3,67±0,15	2,9±0,21	3,49±0,18	3,11±0,3	3,27±0,32
CT, IU/ml	28,9±2,0	26,19±2,6	17,13±4,7	17,45±4,6	18,93±5,1	19,66±5,4	183,4±29,1	43,2±6,3
Ca, mg%	2,55±0,1	2,65±0,1	2,13±0,09	2,53±0,04	—	3,25±0,18	2,15±0,07	3,42±0,16

jacent to the wound surface, the formation of concentrations of epithelial cells with signs of relative dedifferentiation [8], proliferation of gland cells, and hypertrophy of the follicular epithelium over the whole volume of the gland. Higher values of mitotic activity were found among cells in the zone of injury. The intensity of function of the remaining follicles was increased, but the plasma hormone levels remained below those in intact animals.

On the second day the number of DNA-synthesizing cells in the zone of resection was 8.512 ± 0.93% (compared with 3.453 ± 0.42% in the control). Hypertrophy of the thyrocytes (from 4.5 ± 0.12 to 6.5 ± 0.21 μ; $P < 0.005$) and their ultrastructures, and activation of proliferative and morphogenetic processes throughout the gland were observed on the 5th day after resection (Fig. 1a-c). An increase in the number of dividing cells in the organ as a whole was observed (Table 1). Proliferation of the epithelium, subsequent differentiation of the thyrocytes, and new follicle formation in the zone of resection lead to the development of "new" tissue in this part of the organ, but the parameters of its volume and structure did not reach values necessary for complete compensation of the missing part of the gland (Table 1). In the remaining (intact) zone not only did the thyrocytes undergo hypertrophy, but an increase was observed in the mitotic index (MI) of the glandular cells of follicles of all sizes, with the formation of new follicles by fragmentation and division of existing ones. It can accordingly be concluded that TG in rats regenerates by regenerative hypertrophy.

The resected rat TG was under marked functional stress. The iodine-accumulating capacity of the gland, as an indicator of the inorganic phase of iodine metabolism, on the 7th-8th day was 121.7% of the level of radioactive iodine uptake by the glands of intact animals, and the serum T_3 and T_4 levels exceeded control values (Table 2). The relative weight of the gland was restored toward the end of the 3rd week after resection.

Comparison of the T_3 and T_4 levels with the endogenous TTH concentration and the histologic picture of the gland showed that the resected gland was under functional stress; the maximal level of the specific pituitary hormone and, correspondingly, of the thyroid hormones was observed 24 h and 5 days after resection (Table 2). Marked signs of hyperfunction were clearly visible in sections of the gland at these times: hypertrophy of thyrocytes, an increase in the size and number of microvilli in the apical part of the cells, dilatation of capillaries of the cytoplasmic reticulum, and vacuolation and a significant reduction in bulk density of the colloid. Toward the end of the experiment (30 days) the parameters of TG function were still not fully restored to normal.

The study of regeneration of C cells after resection of TG showed a tendency for their number (counted per field of vision) to increase on the 5th day (Table 1), and the increase became significant on the 15th day; the number of C cells increased both in the wall of the follicles and in the parafollicular tissue. It is at this time that maximal release of CT into the blood stream takes place, and its concentration was more than 5 times higher than in the control group. Hypertrophy of the C cells was observed histologically; mitotically dividing and binuclear cells were found among them (Fig. 2a), evidence of marked proliferative changes in this popula-

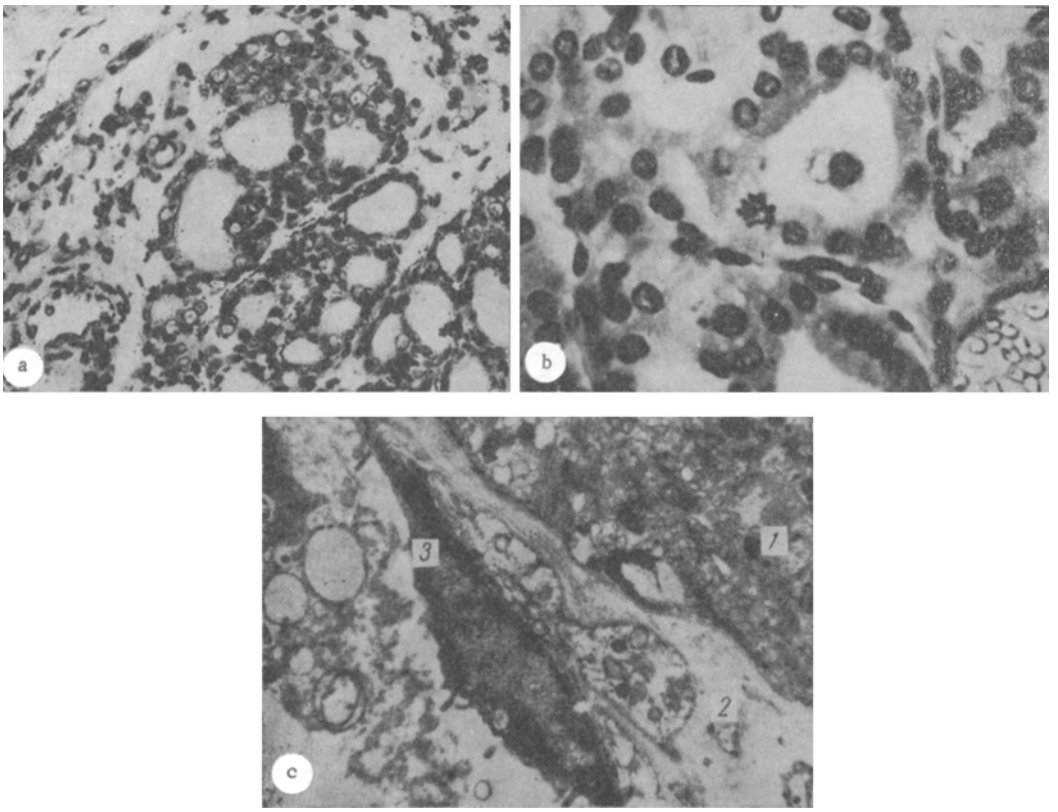

Fig. 1. Morphology of TG on 5th day after resection. a) DNA-synthesizing cells in wall of follicles and epithelial concentrations in zone of resection. Autoradiograph, counterstained with Mayer's hematoxylin, 200×; b) mitotically dividing thyrocyte in wall of small follicle of "intact zone." Hematoxylin and eosin, 400×; c) contact between basal part of thryocyte (1) and capillary. Perivascular space (2) widened. Endothelial cell with numerous microvilli (3), 10,000×.

tion, and confirming their ability to reproduce themselves. Submicroscopically, marked signs of extrusion of secretory material were observed in the cytoplasm of the C cells (Fig. 2b, c). Consequently, definite correlation is observed between the morphological and functional parameters characterizing the C cells. In the later stages the stress reaction still remained, as was confirmed by the significantly raised serum CT level.

The C cells, which produce CT, are known to regulate the blood Ca level. Comparison of serum Ca levels in intact and thyroidectomized rats showed that the Ca balance of the animal after resection of TG was relatively stable (Table 2). At the time of maximal release of CT into the blood stream (15 days) the Ca level was minimal, but at other times of observation regulators of Ca homeostasis were functioning, i.e., the reserve TTH pool of the parathyroid glands was activated. Thus 24 h and 30 days after resection of TG the serum PTH level was 2.16 ± 0.1 and 2.21 ± 0.1 IU/ml respectively compared with 6.4 ± 0.6 IU/ml in the control.

The experimental results showed that during the period of maximal functional stress on the C cells (15 days) the serum T_3 and T_4 levels were low. These data indicate participation of the parafollicular cells in regulation of thyrocytes and confirm their system-forming role [3]. Morphologically, signs of hypothyroidism were found in the thyrocytes in TG of animals killed 15 days after resection: proliferation of connective tissue, a decrease in height of the follicular cells, and leveling of the microvilli.

Thus the follicular thyrocytes and C cells take part in the development of repair processes in the resected rat TG aimed at restoring the normal functional parameters of the gland. Evidence of thyrocyte regeneration – increased proliferative activity, hypertrophy of the cells and ultrastructures – were most marked on the 5th day after resection. As regards the populations of parafollicular cells, their maximal concentration (per field of vision) and hypertrophy and the greatest rise in CT level were observed on the 15th day.

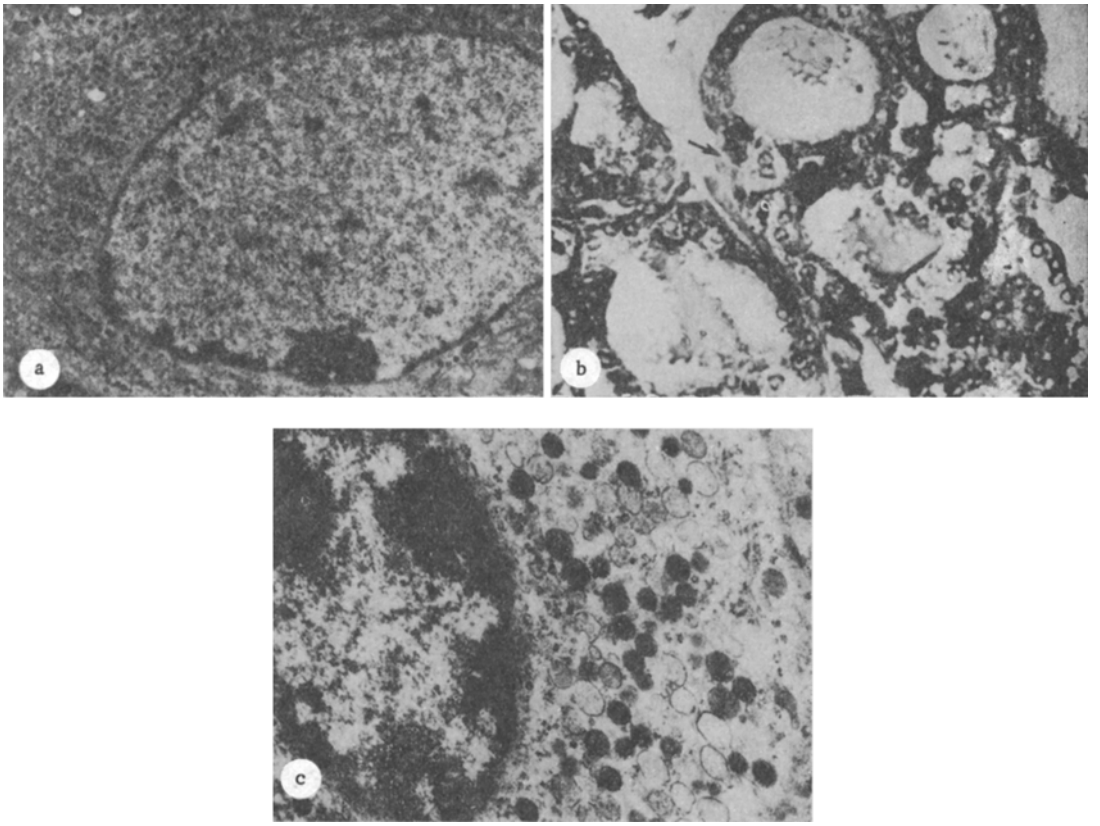

Fig. 2. Histologic and electron-microscopic features of C cells in regenerating TG. a) C cell of intact rat: Cytoplasm filled with electron-dense secretory granules containing CT, large nucleus, 4200×; b) binuclear parafollicular cell (arrow) on 15th day after resection. Impregnation by Grimelius' method, 200×; c) secretory granules in cytoplasm of C cell, with varied electron density (15th day after resection), 10,000×.

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