Effect of Corticosteroids on Argyrophilic Premedullary Thymocytes

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Cells in the premedullar zone of the thymus contain serotonin and catecholamines and show argyrophilicity in the Grimelius test. Their cytoplasm is packed with granules. Glucocorticoids (hydrocortisone) provoke an increase in the number of argyrophilic premedullary cells and in their content of serotonin and catecholamines. Mineralocorticoids induce a prolonged (1-14 days) increase in the number of argyrophilic premedullary cells, enhance their degranulation, and increase their contents of serotonin (2-3 fold) and catecholamines.

Key Words: *argyrophilic premedullary thymocytes; serotonin; catecholamines; hydrocortisone; deoxycorticosterone acetate*

Serotonin and catecholamines (CA) are important neurohumoral factors regulating the function of lymphatic tissues and organs [3,4]. These compounds were found in the aminocytes, specific cells in the thymic premedullary zone [3]. The nature of these cells so far remains unknown. Some researchers believe that aminocytes are APUD cells [1,6,11], while others claim that they are macrophages/APUD cells [7].

We have found that monoamine-containing cells in the premedullary zone of rat thymus can be stained by the method of Grimelius [10]. This is a conventional technique for the identification of APUD cells. However, it was never used for the investigation of rat thymus. Thymus is highly sensitive to adrenal hormones, therefore, it was interesting to examine the effects of glucocorticoids (hydrocortisone), which are known to inhibit lymphopoiesis and immunogenesis [2,9], and those of mineralocorticoids (DOCA), which stimulate these processes, on argyrophilic premedullary cells (APC), specifically, on their count, localization, structure, and changes in the serotonin and CA contents.

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MATERIALS AND METHODS

Experiments were performed on 50 outbred male albino rats weighing 160-200 g. Control group included 20 rats. Two series of experiments were carried out. DOCA (10 mg/kg intramuscularly) was injected in the first series (n=25), and hydrocortisone (10 mg/kg intramuscularly) was injected in the second series (n=25). The animals were decapitated under light ether anesthesia on days 1, 3, 7, and 14 after administration of these hormones. Part of the thymus was fixed in Bouin's fluid, embedded in paraffin, and 5-μ think sections were prepared. The sections were used in the Grimelius test with our modifications (optimization of fixation and exposure times). The size of APC was measured with an ocular micrometer [9].

Serotonin and CA were identified on 7-μ thick cryostat sections which were treated with 2% glyoxylic acid as described [10]. Microspectrofluorimetry was carried out in a LYuMAM-I1 microscope at an output voltage of 1900 V and magnification 200. The serotonin and CA contents were measured using a probe 0.2 mm in diameter and interference filters (525 nm for serotonin and 480 nm for CA). The intensity of fluorescence was expressed in arbitrary units [5].

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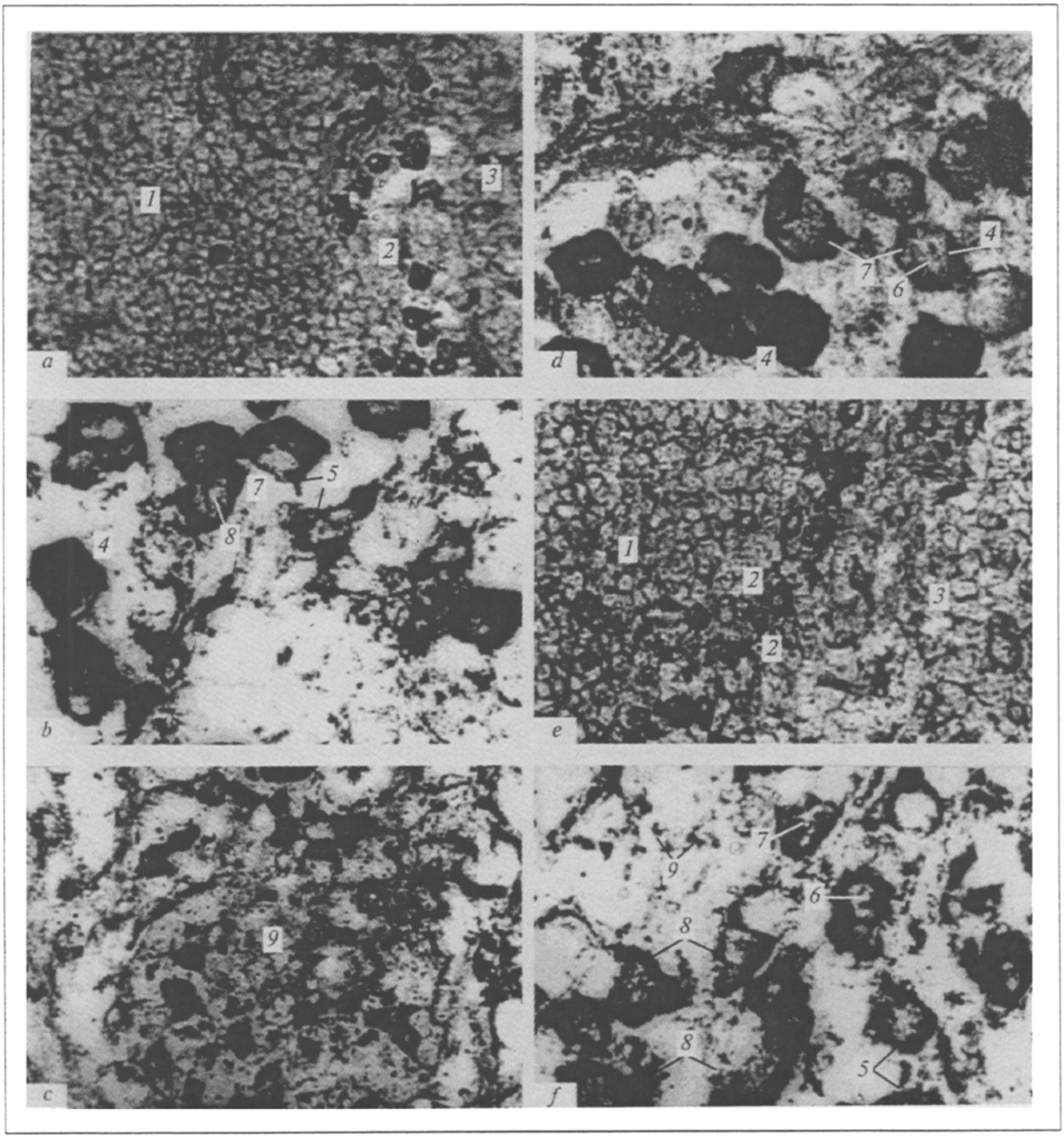

Fig. 1. Argyrophilic premedullary cells of the thymus of intact rats (*a*, *b*) and rats treated with hydrocortisone (*c*, *d*) and the mineralocorticoid DOCA (*e*, *f*). Staining by the method of Grimelius; *a*, *c*, *e*) ×280; *b*, *d*, *f*) ×700. *1*) cortex; *2*) premedullar zone; *3*) medulla; *4*) round argyrophilic cells; *5*) argyrophilic cells with processes; *6*) nucleus; *7*) cytoplasmic granules; *8*) degranulating argyrophilic cells; *9*) granules in the extracellular space.

One hundred measurements of each parameter for each time point were performed and processed using a Statgraphics software. The analysis included construction of histograms and calculation of arithmetic mean, standard deviation, and coefficients of dispersion and asymmetry.

RESULTS

Generally, argyrophilic (Grimelius-positive) cells in the premedullary zone of the thymus of intact animals were arranged in 1-2 rows; sometimes occasional individual cells were seen (Fig. 1, *a*). These cells

TABLE 1. Serotonin and CA Contents in APC of the Thymus After Administration of Corticosteroids

Period after administration	Serotonin			CA		
	$M_0 \pm \sigma_x$, arb. units	dispersion coefficient	asymmetry coefficient	$M_0 \pm \sigma_x$, arb. units	dispersion coefficient	asymmetry coefficient
Norm	2.00±0.69	0.310	+0.56	1.00±0.38	0.330	+0.55
Hydrocortisone:						
day 1	3.20±0.85	0.267	+0.28	1.40±0.38	0.276	+0.18
day 3	3.55±1.01*	0.283	+0.01	1.50±0.34*	0.226	+0.66
day 7	4.14±1.02*	0.248	+0.13	1.53±0.47	0.309	+0.78
day 14	3.25±0.77	0.237	+0.14	1.65±0.37*	0.223	+1.16
DOCA:						
day 1	2.87±0.90	0.313	+0.24	1.68±0.42	0.252	-0.33
day 3	6.24±1.61*	0.393	+0.32	1.66±0.35*	0.209	+0.47
day 7	4.15±0.97*	0.235	+0.18	1.92±0.46*	0.241	+0.05
day 14	4.42±0.97*	0.219	+0.12	1.85±0.47*	0.254	+0.11

Note. $M_0 \pm s_x$ is the arithmetic mean±standard deviation. *$p<0.05$ *vs.* intact cells (Student's *t* test).

varied in size. They were round or had processes; they contained a light nucleus with 1 or 2 nucleoli, and their cytoplasm was filled with granules (Fig. 1, *b*). The mean number of cells per 10 fields of view was 85±11, the mean cell area was 43±18 μ^2. The area of the majority of APC (67%) varied from 30 to 50 μ^2, the area of 20% of APC was 60-110 μ^2, and that of 13% of APC was 10-20 μ^2. The serotonin content varied from 1 to 5.8 arb. units. The mean intensity of fluorescence was 2±0.7 arb. units. In the majority of APC (68%), the intensity of the serotonin fluorescence was 1.6-3.4 arb. units, in about 10% of APC it was 1.0-1.6 arb. units, and in about 6% of these cells it was >4.6 arb. units. The CA content of APC varied from 0.1 to 2.4 arb. units, the mean intensity of fluorescence was 1±0.4 arb. units. In the majority of APC (75%), the intensity of CA fluorescence varied from 0.4 to 1.6 arb. units, in 14% of APC it varied from 1.6 to 2.0 arb. units, and in 11% of cells from 0.6 to 0.4 arb. units. The coefficients of dispersion and asymmetry were similar for both metabolites (Table 1).

Hydrocortisone significantly increased the number of APC to 178±13 on day 3 after injection. The majority of cells were small and located predominantly in the premedullar zone, and the number of Grimelius-positive cells in the cortical and subcapsular zones was increased. Some APC were swollen, and their granules were seen in blood vessels and extracellular space. On days 7-14, the border between the cortex and the medulla became indistinct; APC were identified in cortical and medullar zones, and numerous granules were present in the extracellular space (Fig. 1, *c*). Some APC in the subcapsular zone contacted with mast cells and nerve terminals. Numerous round-shaped APC were packed with granules (Fig. 1, *d*), and APC with degranulated cytoplasm were seen. On days 1, 3, and 7, the APC area was increased (61-66 μ^2). On days 7-14, a tendency toward an increase in the number of APC was observed (264±24). On day 14, the mean size of APC tended to decrease (41±21 μ^2).

A significant increase in the serotonin content was recorded on days 3 and 7 after administration of hydrocortisone (Table 1), the coefficients of dispersion and asymmetry being lower compared with the control on days 1, 3, 7, and 14. A significant increase in the CA content was observed after 3 and 14 days, which was paralleled by a decrease in the coefficient of dispersion and an increase in the coefficient of asymmetry.

The APC count was increased on days 1, 3, 7, and 14 after administration of DOCA, the maximum value (106±31) being recorded on day 14. The cells were located in the premedullary zone, and some of them were swollen and degranulated. Occasional APC were seen in the cortical and medullar zones; occasional binuclear cells were found on day 1. On days 3-7, numerous small, large binuclear, and degranulated APC were seen. In the medullary and subcapsular zones, the number of APC contacting with mast cells and nerve terminals increased. Fourteen days after injection of DOCA, the structure of thymic lobules was preserved; however, the number of small blood vessels and epithelial canaliculi in the medulla increased considerably. The mean size of APC gradually decreased, reaching the value of 41±21 μ^2 by the 14th day. Argyrophilic cells predominated in the premedullar zone, occasional APC were present in the medullar and cortical zones (Fig. 1, *e*), and the majority of APC were degranulated and had processes (Fig. 1, *f*).

On days 3, 7, and 14, the fluorescence of serotonin and CA increased significantly, while the coefficients of dispersion and asymmetry tended to decrease (Table 1).

From our findings it can be concluded that premedullary thymocytes display a number of characteristics common with APUD cells: they are argyrophilic in the Grimelius test, their cytoplasm is packed with granules and contains fluorogenic amines (serotonin and CA) that are involved in the regulation of lymphopoiesis. The APC population is heterogeneous in size and content of serotonin and CA, which indicates its high functional activity. The APC population responds to hydrocortisone and DOCA by changes in cell number, localization, and size as well as in the intensity of production and release of serotonin and CA.

Thus, APC are involved in the realization of the effects of corticosteroids on lymphopoiesis in the thymus.

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Transplantation of Embryonal Pancreas into the Salivary Gland of Adult Rats

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Fragments of embryonal rat pancreas are grafted into the submandibular salivary gland of adult rats with alloxan-induced diabetes. The grafts are not rejected within a 4-week period and contain functionally active insulinocytes. Active function of the endocrine pancreatic fragments is confirmed by a significant increase in the blood content of immunoreactive insulin.

Key Words: *diabetes mellitus; transplantation; insulinocytes; insulin*

Transplantation of the endocrine pancreas is one of the methods employed in the treatment of diabetes mellitus. Isolated islets or free B cells have been transplanted intraperitoneally, under the renal and hepatic capsule [1,5], intraportally [2], or subcutaneously in special capsules [4]. Although these methods hold good promise, transplantation of endocrine tissue remains an operation involving a high risk of the graft-versus-host reaction [3]. High probability of graft rejection prompts the improvement of immunosuppressive therapy and appropriate choice of the sites in the recipient's body where the graft will be effectively established.

Our preliminary studies showed that the salivary glands may be the site where prolonged persistence of transplants of different origin is possible. In order

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