AUTORADIOGRAPHIC AND MORPHOLOGICAL EVIDENCE
OF THE STATE OF THE THYMUS AFTER ADRENALECTOMY,
CASTRATION, AND THYROIDECTOMY

Z. A. Kabolova and A. P. Popov

UDC 616.45+616.681+616.441]-089.87-07;616.438-07

Castration and bilateral adrenalectomy cause hypertrophy of the thymus in rats and increase the incorporation of thymidine-H³ into the nuclei of epithelial cells. Adrenalectomy also leads to an increase in the incorporation of glycine-H³ into cells of the medullary layer. Castration, on the other hand, reduces the intensity of incorporation of labeled glycine. An increase in the incorporation of glycine-C¹⁴ into the epithelial cells is observed in thyroidectomized rats, indicating active protein synthesis.

Connections between the thymus and adrenals, gonads, and thyroid have recently attracted considerable attention in the literature. Conflicting results regarding the state of the thymus in the absence of corticosteroids and sex and thyroid hormones have been obtained [1-6, 8, 9, 12-14]. In these investigations attention was concentrated on description of the morphological picture of the thymus. Only a few studies of metabolism in the thymus after adrenalectomy have been undertaken [7, 10, 11]. However, no information could be obtained on changes in protein and nucleic acid metabolism in the thymus after castration and thyroidectomy.

It was accordingly decided to undertake a morphological and autoradiographic investigation of the thymus after adrenalectomy, castration, and thyroidectomy, using labeled precursors of protein and nucleic acids, in order to shed light on this problem.

## EXPERIMENTAL METHOD

Experiments were carried out on 82 male albino rats weighing 90-100 g. The animals were divided into 3 groups (1 - adrenalectomy, 2 - castration, and 3 - thyroidectomy), all receiving the ordinary diet. Throughout the experiment the adrenalectomized rats were given 0.9% NaCl solution to drink. The animals were sacrificed 2 weeks after the operation, and the thymus was removed and fixed in Carnoy's fluid. After histological treatment the paraffin sections were stained with hematoxylin-eosin, Heidenhain's azan, and for glycoproteins, and the Feulgen reaction was carried out for DNA.

For the autoradiographic studies, thymidine- $H^3$  was injected intraperitoneally into the adrenalectomized and castrated rats 1 h before sacrifice in a dose of 1  $\mu$ Ci/g body weight. In the other series of experiments the adrenalectomized and castrated rats received an injection of glycine- $H^3$  in a dose of 2  $\mu$ Ci/g body weight 3 h before sacrifice. The thyroidectomized rats were injected intraperitoneally with glycine- $C^{14}$  1 h before sacrifice in a dose of 0.5  $\mu$ Ci/g body weight. After fixation in Carnoy's fluid, the tissue of the thymus was treated by the usual autoradiographic method. Sections were coated with type R (NIKFI) liquid nuclear emulsion. After exposure for 1-2 weeks at 4°, the developed preparations were

Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 2, pp. 88-90, February, 1971. Original article submitted August 5, 1970.

© 1971 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00.

TABLE 1. Changes in Relative Weight of the Thymus 2 Weeks after Adrenalectomy, Castration, and Thyroidectomy  $(M \pm m)$ 

Anim <b>al</b> group	No. of animals	Wt. of thymus (in mg/ 100 g body wt.)	P
Control	10	225±24	<0,001
Adrenalectomy Control	10	511±51 202±13	<0,001
Castration Control	13	371±34 213±12	<0,001
Thyroidectomy	9	126±15	

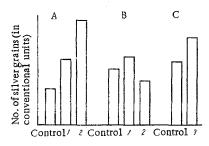


Fig. 1. Incorporation of thymidine-H<sup>3</sup>(A), glycine-H<sup>3</sup> (B), and glycine-C<sup>14</sup> (C) into epithelial cells of thymus 2 weeks after adrenalectomy (1), castration (2), and thyroidectomy (3).

epithelial cells of the medullary layer.

stained with hematoxylin and eosin. The number of grains of reduced silver were counted in 60 fields of vision under a magnification of 300 times. The numerical results were analyzed by statistical methods.

## EXPERIMENTAL RESULTS

In 2 groups of animals (castration and adrenalectomy) an increase in the weight of the thymus and widening of its cortical layer were observed (Table 1). The number of mitoses in the lymphocytes of the cortical layer was increased.

Hypertrophy of the epithelial cells and an increase in the size of their nuclei, enlargement of the Hassall's corpuscles, and an increase in the intensity of their reaction for glycoproteins were observed in the medullary layer. Consequently, the absence of sex hormones or adrenal hormones from the body leads to an increase in activity of the thymus cells, especially of its epithelial cells.

The autoradiographic investigation of the thymus in these groups of experimental rats revealed stimulation of synthesis, as shown by the intensity of incorporation of labeled precursors: thymidine-H<sup>3</sup> and glycine-H<sup>3</sup>. Contrast autoradiographs of the thymus in castrated and adrenalector mized animals revealed a more intensive incorporation of labeled precursors than in the control, while the content of radioactive label in the cortical layer was higher than in the medullary layer. Increased activity of lymphoid cells of the thymus under similar conditions has been described previously [1, 3], and for this reason attention in this investigation was concentrated on activity of the

Quantitative analysis of autoradiographs of the thymus (medullary layer) showed that incorporation of thymidine- $H^3$  in adrenal ectomized rats was increased to  $303 \pm 44$  compared with the control (157  $\pm$  12), and the value after castration was  $470 \pm 43$  (P < 0.01; Fig. 1). The level of protein metabolism in the medullary layer of the thymus was estimated from the incorporation of glycine-H3: 262 ± 24 in the controls and 318 ±5 in the adrenalectomized animals (P < 0.05). Meanwhile, after castration the incorporation of glycine-H<sup>3</sup> in the medullary layer was significantly reduced to 206 ± 8 (Fig. 1). Consequently, after adrenalectomy, increased incorporation of thymidine-H3 and glycine-H3 into the thymus was observed, possible confirmation of the hypothesis that protein and nucleic acid metabolism is stimulated after adrenalectomy. However, after castration, in contrast to adrenalectomy, incorporation of glycine-H3 was reduced. This can evidently be explained by the absence of endogenous sex hormones, with their anabolic stimulating action on protein synthesis. The raised level of incorporation of thymidine-H<sup>3</sup> in the thymus of the castrated rats is evidence of their intensified DNA metabolism.

After thyroidectomy, a decrease in the weight of the thymus was observed (Table 1). Histological sections through the gland showed a decrease in width of the cortical layer, the appearance of pale areas in its parenchyma, and a decrease in the number of lymphocytes and in the number of mitoses. The increase in size of the nuclei of the epithelial cells, the increase in intensity of staining of their cytoplasm for glycoproteins, and the increase in size of the Hassall's corpuscles are all evidence of high activity of the epithelium of the thymus in adrenalectomized animals.

The autoradiographic investigation in thyroidectomized rats showed that incorporation of glycine-C<sup>14</sup> was higher (415 ± 34) than in the control (293 ± 30, P < 0.05, Table 1). After thyroidectomy, besides intensive destruction of lymphocytes, hypertrophy of the epithelial cells evidently takes place and the incorporation of glycine-C14 is increased, confirming the high level of protein synthesis in these cells.

The results of the autoradiographic and morphological investigations of the thymus thus showed that adrenalectomy and castration lead to its hypertrophy and to the stimulation of synthesis in the epithelial cells of its medulla. Thyroidectomy reduces the weight of the thyroid and the number of lymphocytes which it contains but stimulates activity of its epithelial cells.

## LITERATURE CITED

- 1. Z. A. Kabolova, Relationship between the Thymus and Glands of Internal Secretion (Adrenals, Pitu-itary, Gonads, Thyroid). Author's Abstract of Candidate's Dissertation, Moscow (1968).
- 2. D. G. Malkina, in: Problems in Regeneration of the Endocrine Glands [in Russian], Moscow (1961), p. 65.
- 3. E. I. Tarakanov and Z. A. Kabolova, Probl. Éndokrinol., No. 1, 96 (1969).
- 4. H. Chiodi, Endocrinology, 26, 107 (1940).
- 5. T. F. Dougherty and A. White, Am. J. Anat., 77, 81 (1945).
- 6. L. Gyllensten, Acta Path. Microbiol. Scand., 56, 29 (1962).
- 7. F. Knutson and P. M. Lundin, Acta Endocrinol. (Copenhagen), 53, 519 (1966).
- 8. S. N. Marder, J. Nat. Cancer Inst., 11, 1153 (1951).
- 9. J. C. Plagge, Anat. Rec., 116, 237 (1953).
- 10. E. A. Pora, V. Toma, M. S. Rosculet, et al., Rev. Roum. Biol. Ser. Zool., 10, 325 (1965).
- 11. W. B. Pratt, S. Edelman, and L. Aranow, Molec. Pharmacol., 3, 219 (1967).
- 12. W. O. Reinhardt, Ann. New York Acad. Sci., 113, 844 (1964).
- 13. I. Ringler and J. Haynes, Proc. Soc. Exp. Biol. (New York), 111, 606 (1962).
- 14. H. C. Stoerk, Endocrinology, 34, 329 (1944).