MORPHOGENESIS OF INVOLUTION OF THE THYMUS IN OXYTHIAMINE-INDUCED HYPOVITAMINOSIS B_1

G. V. Kovalevskii and G. A. Skryabina

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Lymphopoiesis in the thymus is sensitive to starvation and to qualitative disturbances of nutrition, including deficiency of vitamins of the B group [8]. In alimentary hypovitaminosis B_1, disturbances due to starvation are superposed on the specific vitamin-dependent changes. The former can be reduced to a minimum by using a model of oxythiamine (OT) hypovitaminosis, when the antimetabolite OT quickly inhibits the most important coenzyme function of thiamine [3, 4]. In this connection the morphological manifestations of OT-induced hypovitaminosis are much less marked than in the alimentary form, even in a target organ such as the heart [5]. The exception to this rule is the thymus. The present writers have found that atrophy of the thymus is a constant and the earliest macroscopic sign of OT pathology.

This paper describes the results of a study of morphometric changes in OT-involution of the thymus and some data on its pathogenesis.

EXPERIMENTAL METHOD

OT was injected subcutaneously into noninbred albino rats of both sexes daily in doses of 12.5 mg/kg for 2 weeks and 20 mg/kg for 3 weeks, or as single or double (at an interval of 48 h) doses of 400 mg/kg. Rats receiving injections of 0.85% sodium chloride solution, balanced as regards volume, number, and time, served as the control. Altogether 180 animals were used. The depth of hypovitaminosis was determined by measuring transketolase activity in the thymus and liver. The thymus was fixed in Carnoy's fluid and paraffin sections were stained with hematoxylin and eosin, toluidine blue, and Schiff's reagent. Lymphopoiesis was estimated by studying mitoses in zones of maximal proliferation (the outer cortex and its cambial layer). This latter layer was taken to be a strip of thymocytes 24-25 μ wide, adjacent to the capsule of the lobules. Mitotic indices (MI) were calculated in promille by counting the number of mitoses in 5000 cells. The histoplanimetric corticomedullary coefficent was determined by weighing separately the outlines of a projection of the cortex and of the medulla on paper.

EXPERIMENTAL RESULTS

After injection of 400 mg/kg OT, the changes of hypovitaminosis develop faster in the thymus than in the liver. Whereas in the thymus (Table 1) inhibition of transketolase activity flattened out on a plateau after only 18 h, in the liver this process took 72 h (55.1 ± 4.7 compared with 143.6 ± 7.4 μmoles in the control; $P < 0.001$). A significant increase in weight of the thymus was found after not less than 2 days, with a maximum on the 3rd-4th day of the acute experiment. Atrophy by one-third was produced by a single injection of 400 mg/kg or by a 14-day course of 12.5 mg/kg OT daily; atrophy by 60% was produced by two injections of 400 mg/kg each or by a 3-week course of 20 mg/kg daily.

The loss of weight was attributable to a decrease in the number of cells in the cortex of the gland whereas their number in the medulla remained stable (about 16,300/mm^2 cross section). With a decrease in the number of thymocytes the thickness of the cortex was reduced and the corticomedullary ratio fell. During atrophy of the organ by 60% the cell density per square millimeter cross-section of the cortex fell from 31,500 to 23,900 (by 24%; $P < 0.001$) and the corticomedullary ratio fell from 2.79 to 1.50 (by 46%; $P < 0.01$). Changes in these indices correlated with each other ($r = +0.680 \pm 0.175$; $P < 0.01$). By correcting the density of the thymocytes for the decrease in the corticomedullary ratio, atrophy based on cell loss could be made to coincide

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TABLE 1. Thymus Transketolase Activity (in μmoles/g tissue/h) after Injection of 400 mg/kg of OT

Index studied	Control	6 h	12 h	18 h	24 h	36 h
Activity (M ± m)	133,9±2,4	105,9±6,9	92,2±4,7	76,8±2,5	80,9±4,5	82,8±2,2
Inhibition, %	—	20,9	31,2	42,6	39,6	38,2
P	—	<0,01	<0,001	<0,001	<0,001	<0,001

TABLE 2. Influence of OT on Effect of Hydrocortisone in Outer Cortex of Thymus (M ± m)

Days	Mitostatic effect				Thymolytic effect		
	No. of mitoses per 0.169 mm² section		inhibition, %	P	pycnotic thymocytes per 0.0256 mm² cross section	increase in disintegration, %	P
	T	OT, hydrocortisone					
Control	74,8±3,7	59,5±4,9	20,5	<0,05	32,0±1,9	—	—
1	56,2±3,6	47,3±2,6	15,8	<0,1	35,2±2,9	10	>0,5
2	38,7±3,7	29,5±3,1	23,8	<0,1	62,7±8,8	96	<0,01
3	53,8±3,8	43,8±2,1	18,6	<0,05	104,3±9,3	226	<0,001
4	76,7±2,4	70,0±5,9	8,7	<0,5	73,1±6,0	128	<0,001

TABLE 3. Changes in MI and Number of Thymocytes in Outer Cortex of Thymus after Injection of 400 mg/kg OT

Days	MI (M ± m)	Inhibition, %	P	No. of thymocytes per 0.0169 mm² of section (M ± m)	Loss of cells	P
Control	14,9±0,8	—	—	523±3,0	—	—
1	10,6±0,6	—28,9	<0,01	527±6,0	—	—
2	8,6±0,8	—42,3	<0,001	449±7,4	78	<0,001
3	13,4±1,0	—10,0	<0,5	403±13,4	124	<0,001
4	18,2±0,3	+22,1	<0,01	421±10,5	106	<0,001

with atrophy by weight. OT-involution of the thymus was thus reduced to loss of thymocytes and a decrease in size of the lobules because of collapse of the cortex. It was this collapse which reduced the true decrease in the number of thymocytes by more than half and which masked depopulation of the cortex in the histological sections. Because of this, inversion of the layers of the gland was observed extremely rarely and was connected with the widespread pycnosis and karyorrhexis of cortical cells (up to 40 pycnotic nuclei in 0.0256 mm² of section). In the overwhelming majority of cases, however, more moderate disintegration of the thymocytes was found, and with the presence of phagocytosed, and not of free, nuclear fragments, against the background of marked activation of the system of cortical PAS-positive macrophages (the "starry sky" phenomen).

The results for mitosis were rather unexpected. MI in the outer cortex of the rats in the chronic experiments, decapitated at different times of day (7 a.m., 1 and 6 p.m.) did not differ from the control. The mean daily MI was 11.97 compared with 11.73 in the control. In the acute experiment MI was significantly reduced only 24 and 48 h after the first injection of 400 mg/kg of OT, and the second injection did not inhibit proliferation. Even in the acute experiment, involution of the thymus thus depended more on intensification of thymocyte disintegration than on inhibition of proliferation. Attention was drawn to the cortisone-like type of disintegration (karyorrhexis after a preliminary stage of pycnosis), which is paradoxical for OT pathology, since OT inhibits the production of adrenocortical steroids and depresses their blood level [1, 2]. The only explanation of this paradox was to assume that OT significantly increases the sensitivity of thymocytes to corticosteroids.

To test this hypothesis a special experiment was undertaken. Two series of rats received 400 mg/kg OT and were decapitated 1-4 days later, but 2 h before sacrifice the animals of one series were given an intraperitoneal injection of hydrocortisone in a dose of 2.5 mg/100 g body weight. This dose and exposure of the hormone (Table 2) led to some degree of mitostatic effect, but this extended equally to both experimental and control animals. Meanwhile, a strong thymolytic action, clearly dependent on the stage of hypovitaminosis, was discovered in the experimental rats (Table 2). Whereas 1 day after injection of OT (Fig. 1a) disintegration of the thymocytes induced by hydrocortisone still remained at the control level (Fig. 1b), on the 3rd day of the experiment (Fig. 1c) it exceeded the control value by 226% and still remained high toward the end of the 4th day. OT thus had no significant effect on resistance of dividing thymocytes to hydrocortisone, but reduced it sharply in mature small thymocytes.

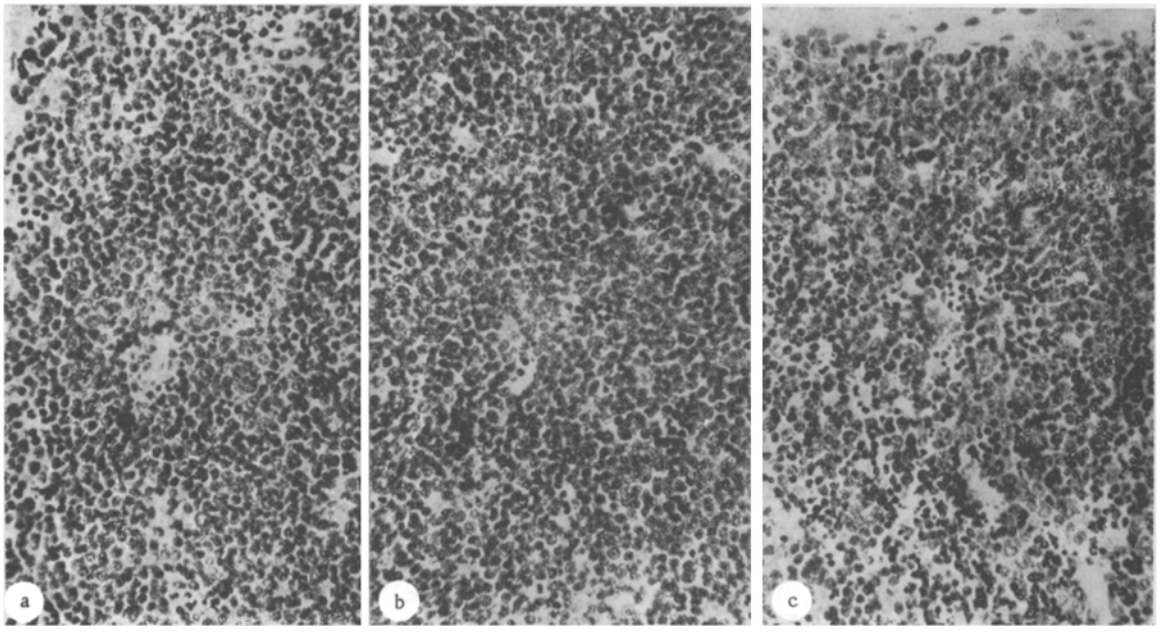

Fig. 1. Thymolytic effect of hydrocortisone (2.5 mg/100 g body weight 2 h before sacrifice) after preliminary administration of OT to rats (40 mg/100 g body weight): a) OT-induced hypovitaminosis for 1 day: small foci of pycnosis and karyorrhexis of thymocytes (capsule of lobule on left); b) control rat: distribution of disintegration of thymocytes comparable with OT-induced hypovitaminosis for 1 day; c) OT-induced hypovitaminosis for 3 days: multiple foci of pycnosis and karyorrhexis with tendency toward fusion against the background of a relative increase in the number of large and medium-sized thymocytes (capsule of lobule above). Hematoxylin and eosin, 280×.

OT inhibited proliferation mainly within the 24-48 h interval. By the 3rd day (Table 3) the MI values were equal again, and on the 4th day MI was higher than in the control. However, this compensation of division did not make good the deficiency of cells, the main course of which, as calculations showed, was the continued disintegration of small thymocytes. If the duration of the mitotic cycle is taken to be 8 h [6, 9], the possible loss of cells on account of inhibition of mitosis on the 2nd, 3rd, and 4th days would be 33, 46, and 44 thymocytes respectively, far less than half their actual loss (Table 3). The rest of the deficit is accounted for by disintegration of thymocytes which cannot be replaced by intensification of proliferative activity. Evidence of the intensity of compensation of disintegration is given by the results of comparison of MI of the outer cortex with MI of the cambium. In the controm MI of the cambium was 30-40% higher than MI of the outer cortex. The same discontinuity between them persisted also during hypovitaminosis in the phase of inhibition of mitosis. However, on the 4th day proliferation increased to such an extent (Table 4) that the difference between the MI values was significantly reduced, and after an additional injection of OT the number of mitoses in the outer cortex actually approached the maximal level for this organ.

OT thus induces involution of the thymus in rats on account of atrophy of its cortex. Morphogenesis of involution can be reduced to two mechanisms: initial temporary inhibition of proliferation and subsequent progressive intensification of disintegration of thymocytes. Disintegration reaches a maximum in the phase of compensation of division, which is not abolished by a second injection of OT. That is why cell disintegration assumes exceptional importance for involution of the thymus in chronic experiments.

TABLE 4. MI of Cambium and Outer Cortex of Thymus 96 h after One and Two Injections of 400 mg/kg OT (M ± m)

Injection of OT	MI of cambium	MI of outer cortex	Difference between MI
Once	20,3±0,9	18,2±0,3	+2,1±1,1
Control	21,6±1,1	14,9±0,8	+6,7±1,3
P	—	<0,01	<0,05
Twice	16,0±1,2	13,1±1,4	+2,4±1,2
Control	17,2±0,8	10,3±0,7	+6,9±1,1
P	—	<0,2	<0,02

Disintegration is due to a decrease in the resistance of small thymocytes to hydrocortisone. The biochemical nature of this phenomenon is not yet clear. It may be associated also with inhibition of enzymes which inactivate hydrocortisone and with an increase in binding of the hormone through slowing of catabolism of specific protein receptors in the thymocytes. Coincidence of the peak of the cortisone-like action of OT with the maximum of hypovitaminosis in the liver (72 h) indicates a contribution of metabolic changes in the liver to thymocyte disintegration. Such a contribution is in agreement with the fact that the thymolytic effect of hydrocortisone is abolished in hepatectomized rats [7].

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ROLE OF NORADRENALIN IN SECRETION OF LUTEINIZING HORMONE

E. I. Adamskaya and V. N. Babichev

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Monoamines, as a class of neuromediators, participate in the regulation of hormonal functions and exert their action through releasing hormones, located in different parts of the CNS and, in particular, in the hypothalamus. Recently considerable attention has been paid to the study of the role of adrenergic mediators in gonadotrophin secretion. The noradrenergic and adrenergic systems of the hypothalamus have been shown to control processes such as the rhythmic type of secretion in ovariectomized rats and the preovulatory liberation of luteinizing hormone (LH) in intact female rats [5, 15].

The writers previously demonstrated correlation between changes in the monoamine content in individual regions of the hypothalamus and the LH level in female rats in various physiological states [1]. However, the results did not give any clear idea of the concrete participation of each monoamine in the regulation of pituitary gonadotrophin function.

The object of the present investigation was to study the role of noradrenalin (NA) in the mechanism of gonadotrophin secretion, specifically: to determine the LH concentration in the pituitary and blood after injection of NA into the preoptic region of the hypothalamus, which is functionally connected with the cyclic liberation of gonadotrophins in female rats [3], and which, as the writers showed previously, is characterized by the greatest changes in NA concentration in the course of the estrous cycle [1].

EXPERIMENTAL METHOD

Experiments were carried out on 120 sexually mature female rats weighing 200-250 g with a stable 4-day estrous cycle. The animals were kept under standard conditions (daylight from 5 to 19 h, temperature 20-23°C).

Laboratory of Physiology of the Endocrine System, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow.

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