EFFECT OF THYMARIN ON MORPHOLOGY AND FUNCTION OF THE MOUSE ADRENAL CORTEX

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In the modern view the biological activity of thymus extracts is connected with the presence in them of certain components of polypeptide nature [10, 9], inducing the competence of the T-system of immunity [7, 8]. The chief regulator of homeostasis of the endocrine functions of the thymus is evidently the adrenals [3], whose hormones (cortisol, corticosterone) have a thymolytic action. The antagonistic character of relations between the thymus and adrenals has been studied mainly from biochemical and immunologic aspects, whereas no morphological studies have been undertaken.

This paper describes a morphological and functional study of the adrenals during stimulation of the cellular and humoral reactions of immunity by means of thymarin, a Soviet preparation obtained from the thymus, the biochemical characteristics of which were described by Morozov [5].

EXPERIMENTAL METHOD

Forty male CBA mice weighing 18-20 g were used. Daily for 10 days the animals were given a subcutaneous injection of 0.1 ml thymarin. The animals were killed on the 1st-10th days of the experiment, three at a time. No thymarin was injected on the day of sacrifice. The control group consisted of 10 mice of the same line. The mice were killed in the second half of the day by transection of the spinal cord in the cervical region. The left adrenal was weighed, frozen with dry ice, and used for histochemical investigation. Activity of NADH- and NADPH-, glucose-6-phosphate- (G-6-PDH), and 3-β-hydroxysteroid- (3-β-HSDH) dehydrogenases and of acid and alkaline phosphatases (AcP and AlP) was determined by the usual methods in unfixed cryostat sections 10 μ thick. Similar unfixed preparations were stained with benzoylated Oil Red O for the detection of lipids. The intensity of the histochemical reaction was estimated cytospectrophotometrically by the plug method, using the FMÉL-1 optical attachment. Measurements were made in monochromatic light at a wavelength of 585 nm and with a probe of constant thickness [4]. The right adrenal, after weighing, was fixed in 10% neutral formalin and embedded in paraffin wax. Sections about 5 μ thick were stained with hematoxylin and eosin, by Van Gieson's and Mallory's method, impregnated with silver by Karupu's method, and treated by the PAS reaction. The linear dimensions of the cells and the volume of the zones of the adrenal cortex and medulla were determined by means of an RA-64U1 drawing apparatus, using the Weibel-Rosival [2] method in the modification of Ariél' and Koval'skii [1].

EXPERIMENTAL RESULTS

The adrenals of the control group of mice were yellowish in color, flattened, round in shape, about 1 mm in diameter, and lay at the anterior end of the corresponding kidney (the right more medially). The cortex was pale yellow with a tinge of gray; the medulla was grayish-brown. The ratio of the weight of the adrenal to that of the kidney was 0.03 ± 0.009. A microscopic study showed that the adrenals consisted of zona glomerulosa, zona fasciculata, and zona reticularis, and also of a medulla, the boundaries of which were clearly distinguishable after impregnation with silver.

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TABLE 1. Linear Dimensions (in μ) of Adrenocortical Cells of CBA Mice (M ± m)

Zona	Thymarin	Control
Glomerulosa	24,36±1,81	32,60±1,85
Fasciculata	25,92±1,36	29,88±1,25
Reticularis	16,65±1,48	12,44±1,32

After injection of thymarin no microscopic changes were found in the adrenals. On morphometric analysis, however, changes were found in the ratio between the different zones of the adrenal cortex. For instance, a very small decrease in the volumes of the zona glomerulosa and zona reticularis was accompanied by a statistically significant decrease in the volume of the zona fasciculata. A significant decrease also was observed in the linear dimensions of the cells in all parts of the cortex (Table 1).

Histological study of sections showed that the adrenal cortex preserved its ordinary structure throughout the investigation. The zona glomerulosa was uniformly developed in all parts of the gland. The boundaries between the cells in this zone were difficult to distinguish. In animals of the control group and in mice killed on the 1st-4th days of the experiment, nests of three-five cells could be distinguished. On the 8th-10 days after injection of thymarin the cells of the zona glomerulosa formed nests consisting chiefly of five-eight cells (sometimes more). The cells in the zona fasciculata of the control animals were arranged as linear trabeculae, directed toward the medulla. The boundaries between the cells were distinct, the cytoplasm pale and sometimes with fine granules of weakly eosinophilic character, and with round nuclei with clear and smooth outlines. Under the influence of thymarin fragmentation of the trabeculae was observed; this was focal in character and took the form of the appearance of groups of two or three cells with pale, structureless cytoplasm. In the zona reticularis of both experimental and control groups the cells were of average size, slightly elongated, and arranged as closely interwoven bands, oriented perpendicularly to the trabeculae of the zona fasciculata. The cytoplasm in the experimental animals, unlike that of the controls, began to stain more intensively as the period of observation lengthened, and PAS-positive material accumulated in it. The cell nuclei, while remaining round or slightly elongated in shape, became sharply hyperchromic.

Cells of the medulla throughout the period of observation were arranged as separate bands or nests, separated by thin layers of connective tissue and large capillaries of sinusoidal type. Cells of the medulla were pale, honeycombed in appearance, with large, intensely stained, and eccentric nuclei.

A marked difference from the control as regards the lipid content in the experimental animals was noted only at the end of observation and was characterized by a marked increase in their content, which was especially marked in the zona fasciculata. In the zona glomerulosa, however, comparatively little sudanophilic material was found at the beginning of the experiment, just as in the control. Cells of the zona fasciculata were loaded with lipids, which were very irregularly distributed. Some cells were filled with large inclusions, others contained many small or medium-sized granules, and in a third group no lipids whatever were found. The total content of lipids decreased in the direction of the zona reticularis, where practically none were found.

Activity of oxidation–reduction enzymes could not be detected at the earliest time after injection of thymarin (1st-4th days). Definite zonality was revealed in their distribution. Granules of diformazan, dark blue in color, were diffusely arranged entirely in the cytoplasm of the cells. Testing for G-6-PDH showed that its activity was highest in the zona glomerulosa and the deep rows of cells of the zona reticularis; activity was appreciably weaker in the zona fasciculata. Activity of NADH- and NADPH-dehydrogenases was maximal in the zona reticularis; activity of NADH-dehydrogenase also was considerable in the zona glomerulosa of the adrenal cortex. Activity of 3-β-HSDH was highest in the zona fasciculata, especially in its upper and middle portions, and diminished gradually in the direction of the zona reticularis. This agrees with the character of distribution of lipids observed in these experiments. At the end of the experiment (9th-10th days) the distribution of oxidation–reduction enzymes exhibited the same zonality, but activity of the enzymes was significantly changed.

For instance, a relative decrease was noted in G-6-PDH activity in the zona glomerulosa and zona reticularis (to 0.780 ± 0.008 relative unit compared with 1.080 ± 0.050 relative units in the control). There was also a significant decrease in 3-β-HSDH activity in the zona fasciculata (down to 0.440 ± 0.004 relative unit compared with 0.880 ± 0.006 relative unit in the control) and in NADPH-dehydrogenase activity in the zona glomerulosa (0.420 ± 0.007 relative unit compared with 0.870 ± 0.008 relative unit in the control).

Experiments thus showed that injection of thymarin is followed by a decrease in the linear dimensions of the cells in the zonal glomerulosa, zona fasciculata, and zona reticularis and also a decrease in the volume of these zones of the adrenal cortex. Considering that an increase in the lipid content and a decrease in activity of oxidation–reduction enzymes are observed at the same time in the cytoplasm of cells of the zona glomerulosa and zona reticularis, it can be concluded that under the influence of thymarin the synthetic function of the adrenals is repressed. This applies evidently above all to the production of proantiinflammatory hormones. This explains the fact that administration of thymarin to patients with chronic inflammatory conditions, burns, trauma, and certain other cases, leads to improvement in the clinical course of the disease [6].

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MITOTIC ACTIVITY OF THE REGENERATING RAT LIVER DURING STIMULATION OF α- AND β-ADRENORECEPTORS

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Previous investigations showed that two injections of propranolol (a β-adrenoblocker) increased the mitotic activity of regenerating liver cells, whereas phentolamine, an α-adrenoblocker, inhibited proliferation [1]. It was suggested that under natural conditions stimulation of β-adrenoreceptors ought to inhibit regeneration, whereas excitation of α-adrenoreceptors should stimulate it. To test this hypothesis experiments were carried out with stimulators of α- and β-adrenoreceptors. It was expected that administration of phenylephrine, a stimulator of α-adrenoreceptors, would intensify regeneration in the liver whereas excitation of β-adrenoreceptors by isoproterenol, on the other hand, would inhibit it.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. About 70% of the weight of the liver was removed from all animals. Some animals were given

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