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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОИ БИОЛОГИИ И МЕДИЦИНЫ
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# BIOGERONTOLOGY

# Tissue-Specific Effects of Peptides

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Synthetic peptides (cytogens) Cortagen, Epithalon, Livagen, and Vilon stimulated the growth of explants from rat brain cortex, subcortical structures, liver, and thymus, respectively, in organotypic cultures. These peptides produced tissue-specific effects: they stimulated the growth of explants from tissues, whose cytomedins (peptide complexes) were used for chemical synthesis.

**Key Words:** *peptides; tissue specificity; tissue culture*

Studies of the mechanisms underlying the regulation of age-related changes in homeostasis by peptides are of considerable importance for modern biological and medical sciences. Recent experiments indicate that aging is associated with suppressed synthesis of regulatory peptide and reduced sensitivity of target cells to regulatory factors, which leads to suppression of various functions in aging organism.

Peptide bioregulators (cytomedins) produce tissue-specific effects on cells, synthesizing these factors. Cytomedins, peptide complexes with a molecular weight of 1-10 kDa, were isolated from various organs and tissues. Cytomedins extracted from the thymus (Thymalin), pineal gland (Epithalamin), brain cortex (Cortexin), prostate (Prostatilen), and retina (Retinalamin) normalize functional activity of the immune and reproductive organs, brain, and retina under pathological conditions and during aging and prolong the life-span of experimental animals [3]. The search for individual peptides, whose biological effects are similar to those of cytomedins, attracts much recent attention.

We used a new methodical approach to the synthesis of peptides with tissue-specific effects [5]. On the basis of amino acid analysis of cytomedins, four prevailing amino acids providing minimum differences between cytomedins were selected. The basic peptide structure X—Glu—Asp—Y was used for chemical synthesis. Theoretical and practical studies performed at the Laboratory of Peptide Chemistry (St. Petersburg Institute of Bioregulation and Gerontology) allowed us to synthesize tetrapeptides Epithalon (Ala—Glu—Asp—Gly), Cortagen (Ala—Glu—Asp—Pro), and Livagen (Lys—Glu—Asp—Ala) and dipeptide Vilon (Lys—Glu). These peptides were named cytogens. After preparative reverse-phase high-performance liquid chromatography the content of test substances was 98-99%. The structure of preparations was confirmed by amino acid analysis and proton magnetic resonance.

Here we studied the effects of Cortagen, Epithalon, Livagen, and Vilon on cell growth in organotypic cultures of rat brain cortex, subcortical structures, liver, and thymus.

## MATERIALS AND METHODS

Experiments were performed in collaboration with N. I. Chalisova. We used 600 explants of the cortex, subcortical structures of the brain (pineal gland area), liver, and thymus from 3-week-old Wistar rats. Culturing was performed as described elsewhere [6]. Fragments of organs (1 $mm^3$) were placed in collagen-coated Petri dishes with nutrient medium consisting of 35% Eagle's medium, 35% Hanks solution, 25% fetal

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bovine serum, and 5% chick embryo extract and supplemented with 0.6% glucose, 0.5 U/ml insulin, 100 U/ml penicillin, and 2 mM glutamine. The test preparations were added in concentrations of 2-200 ng/ml. The effective concentration of synthetic peptides was 20 ng/ml (except for Vilon, 2 ng/ml). Explants cultured without peptides served as the control. Explants were cultured at 37°C for 3 days and examined under a phase contrast microscope equipped with an ocular micrometer. The area index (AI) was calculated as the ratio between the total (together with the area of migrating cells) and initial areas of the explant and expressed in arbitrary units. The results were analyzed by Student's *t* test.

## RESULTS

On day 1 the explants flatten on a collagen substrate. Proliferating and migrating cells formed a growth zone at the periphery of explants. If the diameter of the growth zone increased after 3-day culturing, AI in peptide-treated explants increased compared to that in control samples.

Cortagen, Epithalon, Livagen, and Vilon stimulated the growth of explants from the brain cortex, subcortical structures, liver, and thymus to 140.1±4.7, 127.6±3.1, 121.1±2.5, and 115.7±0.4% of the control, respectively.

Thus, synthetic peptides produced a tissue-specific effect. Our findings indicate that this methodical approach holds much promise for the synthesis of peptides with tissue-specific effects.

The tissue-specific effects of peptides were previously observed in *in vivo* experiments. For instance, Vilon normalized functions of the immune system animals in experimental animals [10]. Combined treatment with Vilon and Epithalon decreased the incidence of tumors, stimulated the antioxidant system, and prolonged the life-span in animals [8]. Studies of the effects of Vilon and Epithalon on the dynamics of irradiation-induced apoptosis in rat splenic lymphocytes showed that they act as the inhibitors of programmed cell death. It should be emphasized that Vilon possesses more pronounced antiapoptotic activity, which confirms the hypothesis on tissue-specific effects of peptide bioregulators [7]. Our findings and published data [1,3,4-9] suggest that these peptides regulate genetic activity and possess tissue-specific genotropic properties. These peptides are probably involved in information exchange between cells. We hypothesized that tissue-specific peptides play an important role in the regulation of the development and specialization of tissues and organs in multicellular organisms [3]. This is consistent with the concept on selective activation of genes as a mechanism of cell differentiation [2].

Studies of functional properties and mechanisms underlying the effect of cytogens would extend our knowledge on their role in the regulation of gene activity.

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