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Saint Petersburg Institute of Bioregulation and Gerontology

Peptides, Genome, Ageing

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Member of the Russian Academy of Sciences

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Interrelation of life style, disease, work, ecology, genetics and biological ageing
Population aged 60 and over in the main world regions
1960 – 2020

Source: UN unit of population
<table>
<thead>
<tr>
<th>Country</th>
<th>Age</th>
<th>Name</th>
<th>Date of birth</th>
<th>Date of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>122</td>
<td>Jeanne Calment</td>
<td>23.02.1875</td>
<td>04.08.1997</td>
</tr>
<tr>
<td>Japan</td>
<td>120</td>
<td>Sigechio Izumi</td>
<td>29.06.1865</td>
<td>21.02.1986</td>
</tr>
<tr>
<td>Russia</td>
<td>117</td>
<td>Semennikova Varvara</td>
<td>10.05.1890</td>
<td>09.03.2008</td>
</tr>
<tr>
<td>USA</td>
<td>114</td>
<td>Martha Graham</td>
<td>12.1844</td>
<td>25.06.1959</td>
</tr>
<tr>
<td>Great Britain</td>
<td>114</td>
<td>Martha Eliza Williams</td>
<td>02.06.1873</td>
<td>02.06.1987</td>
</tr>
<tr>
<td>Canada</td>
<td>113</td>
<td>Pierre Joubert</td>
<td>15.07.1701</td>
<td>16.11.1814</td>
</tr>
<tr>
<td>Spain</td>
<td>112</td>
<td>Joseph Salas Mateo</td>
<td>14.06.1860</td>
<td>27.02.1973</td>
</tr>
</tbody>
</table>
Potential increase in the average human lifespan up to specific limit (biological reserve)

Specific limit of human lifespan - 110-120 years

75-80 years

35-40 years

- the average human lifespan nowadays (premature ageing)
- biological reserve of human life

CHANGES IN THE EXPRESSION AND STRUCTURE OF GENES

DISTURBANCES:
- biorhythms
- water
- food

ADVERSE FACTORS:
- stress
- environmental factors
- radiation

Khavinson V. Peptidergic regulation of ageing (2009)
Technologies and products for longevity

**Scientifically validated**
1. Biorythm
2. Calorie restriction
3. Antioxidants
4. Peptide bioregulators
5. Telomerase activators
6. Physical exercises

**Under investigation**
1. Melatonin
2. Metformin
3. Vitamins
4. Stem cells
1. Peptide bioregulators: biological activity
Over 15 million patients were treated with these pharmaceuticals both for prevention and treatment of different pathological states (1982 – 2015).

<table>
<thead>
<tr>
<th>Preparation (State Pharmacopoeia of the Russian Federation)</th>
<th>Source of the peptides</th>
<th>Patents</th>
</tr>
</thead>
</table>
## Cytogens® - synthetic peptides

(Pharmaceutical and food supplements)

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Correction of functions</th>
<th>Patents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bronchogen®</strong></td>
<td>Bronchopulmonary system</td>
<td>Khavinson V. et al. US Patent № 7,625,870 (2009)</td>
</tr>
<tr>
<td><strong>Cardiogen®</strong></td>
<td>Cardiovascular system</td>
<td>Khavinson V. et al. US Patent № 7,662,789 (2010)</td>
</tr>
</tbody>
</table>
Peptides regulate gene expression in the mice heart.

15247 genes tested, 
maximum increase - 6.61 fold; maximum decrease - 2.71 fold

Euler-Venn Diagrams

Decreased protein synthesis in human cells (different age)

* - p<0.05 as compared to the (20-30);  ** - p<0.05 as compared to the (50-60)
Peptides suppress HER-2/neu oncogene expression in transgenic mice

* - p<0.05 as compared to the control;  ** - p<0.05 as compared to the control

Livagen increases protein synthesis in young and old rat hepatocytes (*standard model*)

- Livagen (liver peptide) increases protein synthesis by +38.6% in young rats.
- Livagen (liver peptide) increases protein synthesis by +172.7% in old rats.

* - p<0.05 as compared to the control

Epitalon increases telomeres length and the limit of fibroblasts division

Increase in the number of cell divisions in the experiment by 42.5% as compared to the control.

* - p<0.05 as compared to the control

Anisimov V., Khavinson V. Biogerontology (2010)
Retinal peptides induce differentiation of polypotent ectoderm of *Xenopus laevis*
Epitalon increases melatonin synthesis in old monkeys

* - p<0.05 as compared to young animals, placebo; ** - p<0.05 as compared to old animals, placebo

Epitalon increases melatonin synthesis in pinealocytes

The expression of pCREB in pinealocytes

Control

Epitalon

immunocytochemistry, x400


Epitalon

pCREB - transcription factor

AANAT – protein (enzyme) aralkylamineN-acetyltransferase

serotonin

AANAT

melatonin

KHAVINSON PEPTIDES®
Peptides inhibit development of carcinogenesis induced in animals

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Animal species</th>
<th>Carcinogenic agent</th>
<th>Tumour</th>
<th>Tumour incidence,%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Peptide</td>
</tr>
<tr>
<td>Epithalamin (pineal peptides)</td>
<td>rats</td>
<td>DMBA</td>
<td>mammary gland</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X-ray irradiation</td>
<td>mammary gland</td>
<td>16</td>
</tr>
<tr>
<td>Thymalin (thymus peptides)</td>
<td>rats</td>
<td>DMBA</td>
<td>mammary gland</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X-ray irradiation</td>
<td>mammary gland</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>C3H mice</td>
<td>X-ray irradiation</td>
<td>leukemia</td>
<td>46</td>
</tr>
<tr>
<td>Thymogen</td>
<td>rats</td>
<td>isotops $^{90}$Sr and $^{137}$Cs</td>
<td>any malignant tumors</td>
<td>16</td>
</tr>
<tr>
<td>Vilon</td>
<td>C3H mice</td>
<td>DMH</td>
<td>kidney</td>
<td>60</td>
</tr>
<tr>
<td>Epitalon</td>
<td>C3H/He mice</td>
<td>MMTV</td>
<td>mammary gland</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>female rats</td>
<td>Constant lighting</td>
<td>mammary gland</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>male rats</td>
<td>Constant lighting</td>
<td>leukemia</td>
<td>12</td>
</tr>
</tbody>
</table>

* - p < 0.05 as compared to the control
Peptides increase average life span (the results of 30 years investigation - 25 experiments)

* - p<0.05 as compared to the control

Khavinson V. Peptidergic regulation of ageing (2009)
Peptide bioregulation of vital activity

Adverse factors (stress, harmful environment, radiation, etc.)

- Decreased gene activity
- Decreased protein synthesis
- Decreased functions of organs
- Decreased vital activity

Pathological processes and accelerated ageing

Recovery

Peptides (small proteins)
### Peptides increase organism vital resource

<table>
<thead>
<tr>
<th>Biological Activity Increase</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein synthesis</strong> in rat hepatocytes - by <em>39-173%</em></td>
<td>Khavinson V. Peptides and ageing. NEL (2002)</td>
</tr>
<tr>
<td><strong>Growth</strong> of animal <strong>cells</strong> by <em>22-42%</em></td>
<td>Khavinson V. Peptides and ageing. NEL (2002)</td>
</tr>
<tr>
<td>The <strong>amount</strong> of heterochromatin in lymphocytes of elderly people by <em>42.4%</em></td>
<td>Khavinson V. et al. NEL (2003)</td>
</tr>
<tr>
<td>The number of divisions of human cells - by <em>42.5%</em> and a <strong>2.4-fold increase</strong> in the average length of <strong>telomeres</strong></td>
<td>Khavinson V. et al. Bul. Exp. Biol. Med. (2004)</td>
</tr>
<tr>
<td><strong>3.1-fold</strong> decrease in <strong>tumor incidence</strong> in animals</td>
<td>Anisimov V., Khavinson V. Biogerontology (2010)</td>
</tr>
</tbody>
</table>
2. Peptide bioregulators: mechanism of activity
Penetration of small peptides (CPPs) into cell

Schematic representation of nucleopore

Penetration of FITC-labeled peptide into HeLa cells


A, C – staining of DAPI nuclei (DNA identification)

B, D – fluorescence

6-hour cell incubation with FITC-labeled peptide (1,2 x 10⁻⁶ M)
The influence of peptides on hydrolysis of fluorescence-labelled deoxyribo oligonucleotide with endonuclease WEN1

Oligonucleotide - (5’) FAM – CGC CGC CAG GCG CCG CCG CG (3’)
(FAM – carboxyfluorescein)

E - Epitalon
(Ala-Glu-Asp-Gly)

B - Bronchogen
(Ala-Glu-Asp-Leu)

C - Cardiogen
(Lys-Glu-Asp-Arg)

P - Pancragen
(Lys-Glu-Asp-Trp)

The structures of peptides (3D models)

Conformations of the peptides **Ala-Glu-Asp-Gly** (Epitalon), **Glu-Asp-Gly** (Chonluten), **Lys-Glu** (Vilon) with optimal minimization energy.

Red colour indicates oxygen molecules, blue – nitrogen molecules, black – carbon molecules, light grey - polar hydrogen molecules. Nonpolar hydrogen molecules are not displayed.
Peptide and DNA (3D models)
Model of DNA-peptide complex

Major groove
5’-GGCAGG-3’
3’-CCGTCC-5’

Pancragen

negatively charged part of DNA
positively charged part of DNA
neutrally charged part of DNA
### Pancragen sites of binding in gene promoter regions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene regulatory region, range -499 to 100 bp</th>
<th>GenBank №</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pdx1</td>
<td>ATCAAATGCTTCTGACCTAGAGCTGGTCGCAAAACTTTTTTTTTTATCGGATATCCTGGCACAACGTTAATATAAAATTTAAAACTCAACAGTGGCAGCGACGGAGAGCTGGGTCTGCAAACTTTTTTTTTATCGTATTCCGCAACAGTTAAATAAAAAATTAAAAACTCAGATGCTCAGCCTGCTCCTGGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG GTACCTGGGCTAGCTTTAGTCCGACCGAGGCAAATACGGGGCCGCTGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG GTACCTGGGCTAGCTTTAGTCCGACCGAGGCAAATACGGGGCCGCTGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG GTACCTGGGCTAGCTTTAGTCCGACCGAGGCAAATACGGGGCCGCTGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG GTACCTGGGCTAGCTTTAGTCCGACCGAGGCAAATACGGGGCCGCTGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG GTACCTGGGCTAGCTTTAGTCCGACCGAGGCAAATACGGGGCCGCTGCAAACTCAGCTGAGAGAGAAATTGGAACAAAAAGCAG</td>
<td>NM_000209.3</td>
</tr>
<tr>
<td>Pax6</td>
<td>GCCAGCGAGCCGCGAGGAGAGCTCGGGACAGC</td>
<td>NM_000280</td>
</tr>
<tr>
<td>Pax4</td>
<td>GCCAGCGAGCCGCGAGGAGAGCTCGGGACAGC</td>
<td>NM_006193</td>
</tr>
<tr>
<td>Nkx2.2</td>
<td>GCCAGCGAGCCGCGAGGAGAGCTCGGGACAGC</td>
<td>NM_002509.3</td>
</tr>
<tr>
<td>Foxa2</td>
<td>GCCAGCGAGCCGCGAGGAGAGCTCGGGACAGC</td>
<td>NM_153675.2</td>
</tr>
</tbody>
</table>

**Red** colour indicates Pancragen sites of binding.
Peptide activates selective gene transcription during its binding with DNA. This can lead to mRNA formation and the synthesis of apoptotic, proliferative and differentiation proteins. This increases cell resource and slows down cellular senescence.

3. Peptide bioregulators: clinical study
Peptides decrease morbidity in «Gazprom» employees working under adverse environment

**Main group** (11,192 employees) received 6 peptides orally for 30 days, to improve functions of the immune system (**Crystagen**), brain (**Pinealon**), blood vessels (**Vesugen**), bronchi (**Chonluten**), liver (**Ovagen**), cartilage tissue (**Cartalax**).

**Control group** (3,000 employees) received multivitamins orally for 30 days.
Human (35-55 years) morbidity decrease

PEPTIDES

2.4 times

2.8 times

Control group

Acute respiratory diseases

Total morbidity

11 192 employees of «Gazprom» received a complex of 6 peptide bioregulators to improve the functional state of immune system, brain, blood vessels, bronchi, liver, cartilage tissue. The observation period - 1 year
The influence of Epithalamin (*pineal peptides*) on survival of elderly patients (*15 years unique clinical study*)

Korkushko O. et.al. (2011)

![Graph showing the influence of Epithalamin on survival rate of elderly patients. The graph compares basic therapy (control) with basic therapy + complex of pineal peptides. There are significant differences marked with asterisks (*p < 0.05*) compared to the control group.](image-url)
Main peptide complexes to enhance human resource and prevent age-related disorders

The scheme of treatment:
1. Pinealon, Vesugen – 10 days
2. Crystagen, Chonluten – 10 days
3. Ovagen, Cartalax – 10 days

Total 30 days

Health assessment is conducted before the application and repeated in 4 months.

This is followed by mathematical processing, statistics and recommendations. Recommended 2 courses each year.
Enhancement of vital resource (following peptide complex) of the Russian Olympic team in rhythmic gymnastics

left to right: A. Shumilova (coach), D. Kondakova, A. Zaripova (coach), J. Lukonina, Prof. V. Khavinson, E. Kanaeva, V. Schtelbaums (coach), I. Viner-Usmanova (main coach of team, honored coach of Russia), O. Buyanova (coach), D. Dmitrieva
Peptide application

- Medications
  - Natural preparations
    - Synthetic preparations
  - Synthetic
- Biologically active supplements
  - Natural
  - Synthetic
- Cosmetology
  - Preventive cosmetology
  - Medical cosmetology
- Veterinary
  - Animal husbandry
    - Aviculture
    - Domestic animals
- Nutrition
  - Functional foods and beverages
    - Sportsmen products
Theoretical, experimental and clinical investigations have shown the role of signal small peptides in epigenetic regulation of gene expression, protein synthesis, life resource and life span increase.
Regulation of gene expression and protein synthesis

Life resource, life span increase
Prof. V. Khavinson and staff of the Laboratory of Biogerontology of the St. Petersburg Institute of Bioregulation and Gerontology
(Left to right, upper row: R. Khalimov, prof. V. Khavinson, prof. T. Kvetnaia, V. Basharina; lower row: S. Tarnovskaya, E. Plotnikova, N. Linkova)
THANK YOU FOR YOUR ATTENTION!

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