

# Effects of Peptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly on Hormonal Activity and Structure of the Thyroid Gland in Hypophysectomized Young Chickens and Old Hens

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Hypophysectomy in 5-days chickens and old hens was followed by hormonal disturbances and structural changes in the thyroid gland. Administration of peptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly synthesized on the basis the amino acid composition of extracts from the anterior and posterior lobes of the pituitary gland, respectively, to hypophysectomized birds for 40 days significantly reduced the degree of these changes. The normalizing effect of synthetic peptides on the concentration of thyrotrophic hormone and thyroid hormones in old hens was less pronounced than in chickens.

**Key Words:** *pituitary gland; peptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly; thyroid gland; hormones*

Our previous studies showed that hypophysectomy in birds of different ages is followed by secondary immunodeficiency, hypercoagulation, and suppression of fibrinolysis. These changes were not observed after administration of the peptides [4-7] synthesized on the basis of the amino acid composition of extracts from the anterior and posterior lobes of the pituitary gland [8,9]. The reactions were shown to differ in neonatally hypophysectomized chicken, adult hens, and old hens.

The pituitary gland is the major regulator of thyroid gland functions. Thyroid hormones produce a strong effect on the immune system and hemostasis [2].

Here we studied the effects of synthetic peptides on functional activity and morphological structure of the thyroid gland in hypophysectomized chickens and old hens.

## MATERIALS AND METHODS

Experiments were performed on 60 young chickens (5 days) and 45 old hens (5 years). Group 1 birds

(15 chickens and 10 old hens) were sham-operated. The remaining birds were hypophysectomized. After hypophysectomy, the chickens ( $n=15$ ) and old hens ( $n=15$ ) of group 2 received physiological saline for 40 days. The birds of groups 3 (15 chickens and 10 hens) and 4 (15 chickens and 10 hens) were treated with Lys-Glu-Asp-Gly (anterior pituitary peptide, APP) and Ala-Glu-Asp-Gly (posterior pituitary peptide, PPP), respectively. The peptides were injected intramuscularly once a day in a dose of 0.1 mg/kg (in 1 ml physiological saline).

Morphological study of the thyroid gland was performed after 1.5 months [1,2]. The concentrations of pituitary thyrotrophic hormone (TTH), triiodothyronine ( $T_3$ ), tetraiodothyronine ( $T_4$ ), and free thyroxin in the blood were measured by ELISA on a DigiScan vertical scanning 8-channel photometer. The study was conducted with "Thyroid EIA-TTH-1", "Thyroid EIA-triiodothyronine-01", "Thyroid EIA-thyroxin-01", and "EIA-free  $T_4$ " kits (AlkorBio).

Experimental data were analyzed by statistical methods for related and nonrelated observations. The significance of differences was evaluated.

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## RESULTS

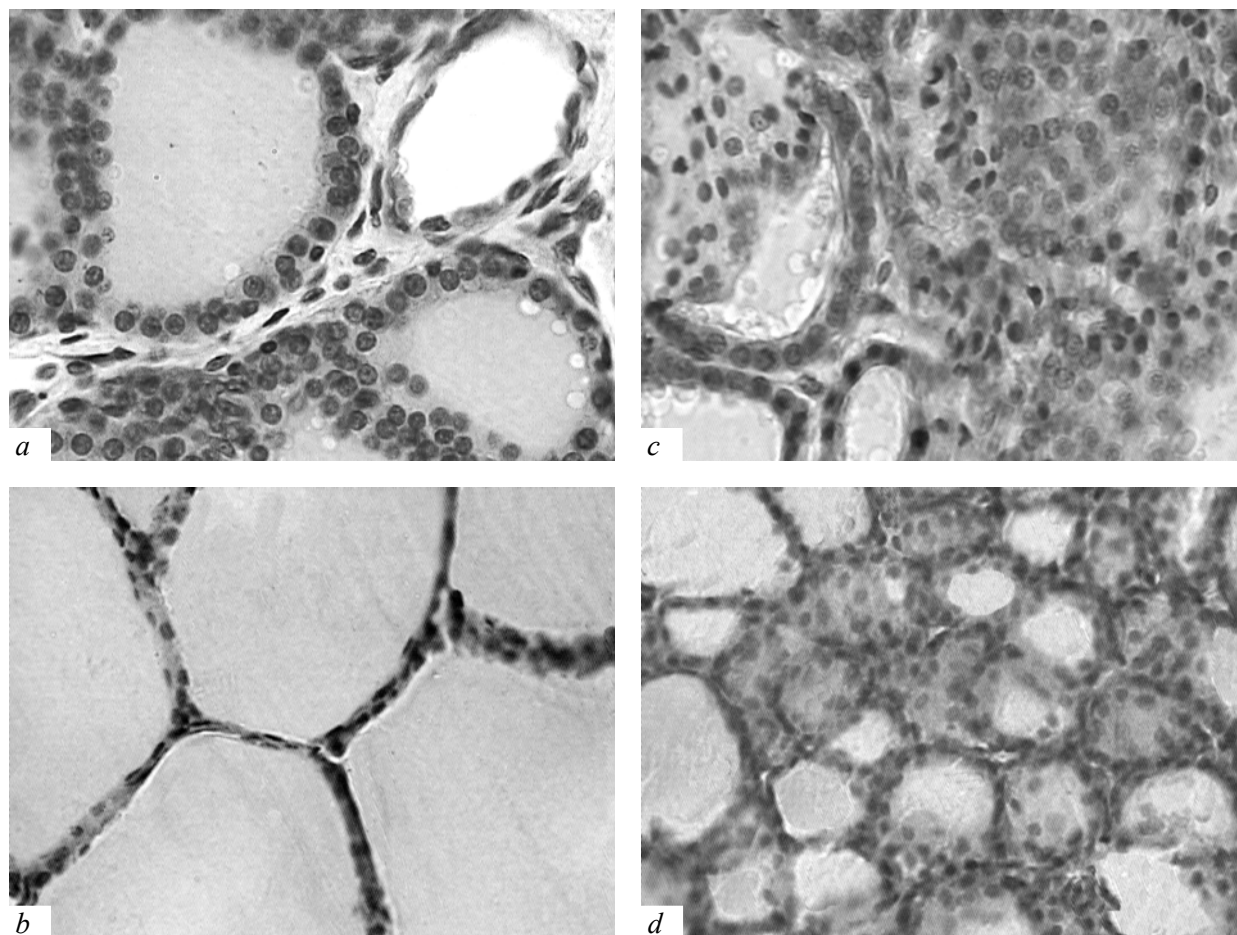
Hypophysectomy in chickens was followed by growth retardation. The birds were characterized by sluggishness, reduced excitability, and anorexia. Hypophy-

sectomy in 5-days chickens and old hens was accompanied by a decrease in body weight by 63 and 31% respectively ( $p \leq 0.05$ ). The weight of the thyroid gland in chickens and old hens decreased on 17 and 30% respectively. The size of follicles increased by 4.5-5.5

**TABLE 1.** Effects of APP and PPP on Morphological Characteristics of the Thyroid Gland in Hypophysectomized Chickens and Old Hens ( $M \pm m$ )

| Parameter                                 | Group 1      | Group 2        | Group 3                 | Group 4                    |
|---|--------------|----------------|-------------------------|----------------------------|
| Mortality rate                            | 0            | 3              | 2                       | 0                          |
|   | 0            | 3              | 0                       | 0                          |
| Body weight, g                            | 296.4±6.2    | 169.8±7.4*     | 237.6±15.9**            | 276.8±12.4 <sup>+o</sup>   |
|   | 1835.2±14.7  | 1267.3±10.3*   | 1438.1±11.8**           | 1754.4±13.7 <sup>**o</sup> |
| %   | 100          | 57.29±2.46     | 80.16±1.99              | 93.39±2.16                 |
|   |              | 69.06±0.56     | 78.36±0.64              | 95.59±0.74                 |
| Absolute weight of the thyroid gland, mg  | 84.2±2.3     | 70.16±1.30*    | 74.36±1.13**            | 76.47±1.25 <sup>+</sup>    |
|   | 349.7±9.6    | 269.1±8.5*     | 322.92±4.65**           | 325.61±3.78**              |
| %   | 100          | 83.3±2.1       | 88.1±1.6                | 90.4±2.0                   |
|   |              | 70.1±1.8       | 92.2±2.5                | 93.1±2.3                   |
| Relative weight of the thyroid gland, mg  | 0.028±0.0007 | 0.041±0.0008*  | 0.031±0.0004*           | 0.027±0.0004 <sup>+</sup>  |
|   | 0.019±0.0005 | 0.021±0.0006   | 0.022±0.0003*           | 0.018±0.0002 <sup>o</sup>  |
| %   | 100          | 146.3±4.6      | 110.7±1.7               | 96.4±1.3                   |
|   |              | 110.5±4.1      | 115.7±3.7               | 94.7±3.5                   |
| Width of the capsule, μ                   | 97.08±6.68   | 77.64±3.2*     | 86.21±4.1**             | 89.6±3.5**                 |
|   | 111.0±4.48   | 88.7±7.8*      | 97.6±4.3**              | 102.4±5.1**                |
| %   | 100          | 79.3±3.5       | 88.6±2.3                | 91.7±1.9                   |
|   |              | 79.2±3.9       | 87.3±2.6                | 91.8±2.4                   |
| Average area of follicles, μ <sup>2</sup> | 921.8±15.8   | 4593.8±137.82* | 298.57±34.7**           | 324.51±16.7**              |
|   | 1205.73±45.9 | 5425.7±176.4*  | 765.87±63.43**          | 698.12±45.21**             |
| %   | 100          | 550.3±19.2     | 32.3±1.8                | 35.1±2.4                   |
|   |              | 450.2±23.3     | 63.4±3.7                | 57.9±3.1                   |
| Shape of follicles                        | 0.85±0.055   | 0.73±0.051*    | 0.81±0.045 <sup>+</sup> | 0.82±0.048 <sup>+</sup>    |
|   | 0.85±0.039   | 0.71±0.021*    | 0.81±0.023 <sup>+</sup> | 0.84±0.031 <sup>+</sup>    |
| %   | 100          | 85.8±3.6       | 95.2±2.1                | 96.4±2.3                   |
|   |              | 83.5±2.3       | 95.3±3.1                | 98.8±2.8                   |
| Height of the epithelium, μ               | 6.78±0.08    | 1.56±0.06*     | 4.97±0.02**             | 5.64±0.04**                |
|   | 6.03±0.04    | 1.87±0.004*    | 5.12±0.01**             | 5.38±0.04**                |
| %   | 100          | 23.0±1.9       | 73.3±2.4                | 83.2±1.6                   |
|   |              | 31.1±2.5       | 84.9±2.1                | 89.2±2.9                   |
| Nuclear-cytoplasmic ratio, units          | 0.52±0.02    | 0.68±0.04*     | 0.57±0.01**             | 0.56±0.03**                |
|   | 0.63±0.01    | 0.67±0.002*    | 0.64±0.001              | 0.65±0.003                 |
| %   | 100          | 130.7±2.8      | 109.6±1.8               | 107.6±1.5                  |
|   |              | 106.3±2.7      | 101.6±2.2               | 103.1±2.7                  |

**Note.** Here and in Table 2: numerator, chickens; denominator, old hens.  $p < 0.05$ : \*compared to group 1, +compared to group 2, °compared to group 3.



**Fig. 1.** Thyroid gland in chickens. Here and in Fig. 2: staining with hematoxylin and eosin,  $\times 400$ . Group 1 (a), group 2 (b), group 3 (c), and group 4 (d).

times. Thyrocytes were flattened. The interfollicular epithelium was undetectable (Table 1, Fig. 1). The chickens exhibited more pronounced decrease in the concentrations of TTH,  $T_3$ ,  $T_4$ , and free thyroxin (as compared to old hens; Table 2, Fig. 2).

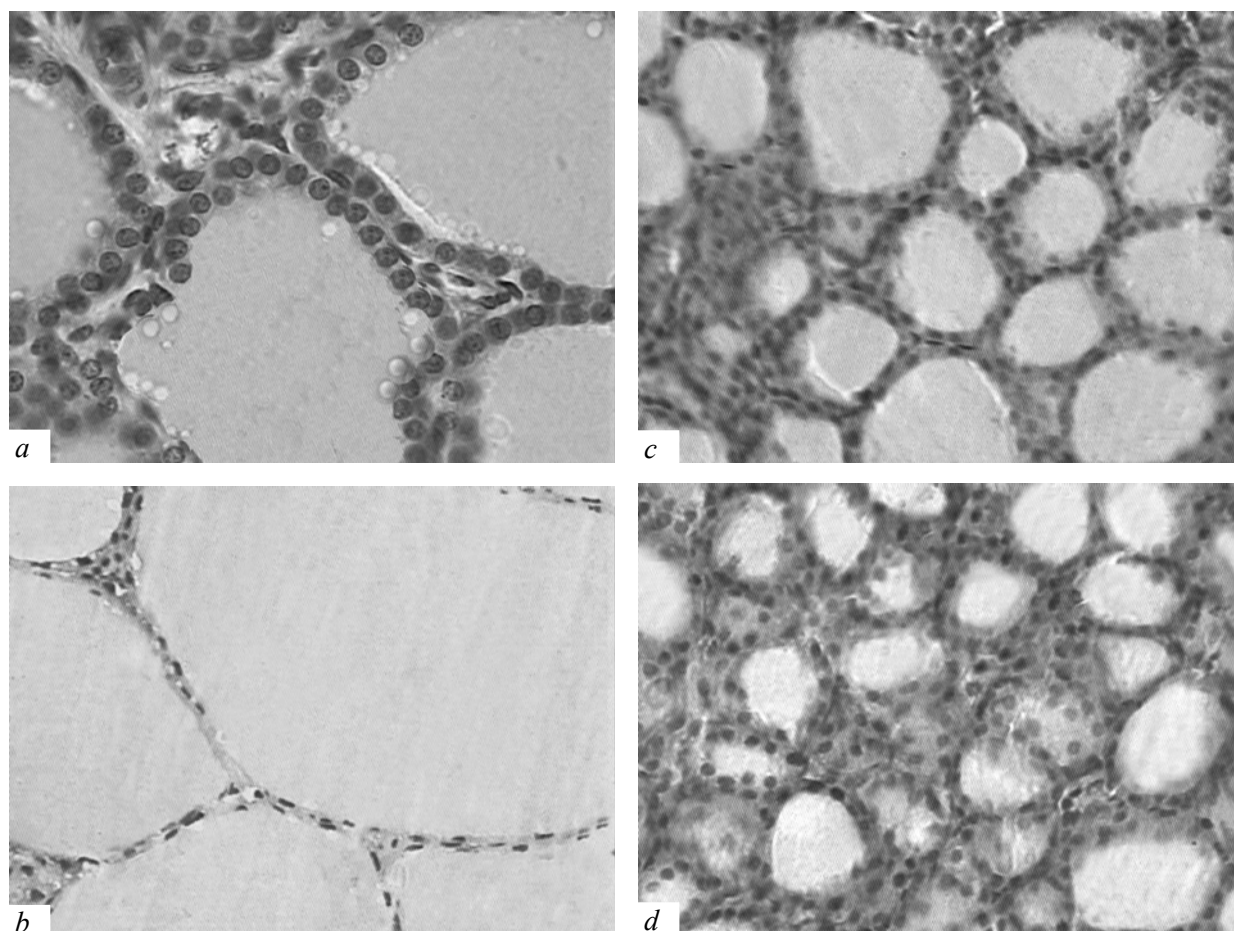
Administration of APP to chickens was followed by a 23% increase in their body weight, while body weight in old hens remained practically unchanged. The weight of the thyroid gland returned to normal in old hens, but slightly increased in young birds (Table 1). APP treatment was accompanied by a decrease in the mean sectional area of follicles (by 15 and 3 times compared to groups 2 and 1, respectively). Microfollicles had a round shape and were covered with the cubical epithelium. Oxyphilic colloid was found in these structures. The mean sectional area of follicles in APP-receiving birds was decreased by 9 and 1.5 times compared to that in specimens of groups 2 and 1, respectively (Table 1).

Administration of APP to young chickens was shown to prevent flattening of the follicular epithelium and colloid accumulation in the follicular cavity. The

interfollicular epithelium (source of new follicles) was well defined between the microfollicles covered with the cubical epithelium. The intermediate follicles were found only at the periphery of some lobules. APP had a moderate stimulating effect on proliferation of epithelial cells in old birds. Therefore, the size of follicles in old hens was greater than in chickens.

The increase in the nuclear-cytoplasmic ratio and decrease in the height of thyrocytes in APP-receiving chickens were less significant than in group 2 birds. APP had a stimulating effect on the growth of the thyroid capsule. However, the width of the thyroid capsule in these birds was lower than in healthy specimens (Table 1). The shape of the epithelium in old birds remained practically unchanged after administration of APP. This treatment was followed by a slight increase in the nuclear-cytoplasmic ratio (Table 1).

Administration of PPP to young and old birds was followed by an increase in body weight (up to the normal value). The weight of the thyroid gland practically did not differ from normal. The size of microfollicles in PPP-receiving chickens was lower



**Fig. 2.** Thyroid gland in old hens.

**TABLE 2.** Effects of APP and PPP on the Concentration of Thyroid Hormones in Hypophysectomized Chickens and Old Hens ( $M \pm m$ )

| Parameter                | Group 1           | Group 2               | Group 3                 | Group 4                 |
|--------------------------|-------------------|-----------------------|-------------------------|-------------------------|
| TTH, $\mu\text{U/liter}$ | $0.146 \pm 0.029$ | $0.0174 \pm 0.0001^*$ | $0.081 \pm 0.001^{**}$  | $0.069 \pm 0.001^{**+}$ |
| %                        | $0.091 \pm 0.016$ | $0.017 \pm 0.0011^*$  | $0.043 \pm 0.0012^{**}$ | $0.036 \pm 0.001^{**+}$ |
|                          | 100               | $11.9 \pm 0.068$      | $55.4 \pm 0.68$         | $47.2 \pm 0.068$        |
|                          |                   | $18.7 \pm 1.2$        | $46.15 \pm 1.3$         | $39.2 \pm 1.09$         |
| $T_3$ , nmol/liter       | $4.24 \pm 0.19$   | $0.57 \pm 0.23^*$     | $1.35 \pm 0.23^{**}$    | $1.24 \pm 0.16^{**}$    |
| %                        | $8.7 \pm 3.22$    | $2.02 \pm 0.1^*$      | $3.43 \pm 0.2^{**}$     | $2.62 \pm 0.4^{**+}$    |
|                          | 100               | $13.4 \pm 5.42$       | $31.8 \pm 5.42$         | $29.2 \pm 3.77$         |
|                          |                   | $23.2 \pm 1.15$       | $39.4 \pm 2.29$         | $30.1 \pm 4.59$         |
| $T_4$ , nmol/liter       | $8.13 \pm 0.25$   | $1.78 \pm 0.19^*$     | $4.41 \pm 0.18^{**}$    | $3.81 \pm 0.21^{**}$    |
| %                        | $15.3 \pm 2.5$    | $4.03 \pm 1.1^*$      | $7.25 \pm 0.1^{**}$     | $6.44 \pm 0.3^{**}$     |
|                          | 100               | $21.8 \pm 2.34$       | $54.2 \pm 2.21$         | $46.9 \pm 2.58$         |
|                          |                   | $26.3 \pm 7.18$       | $47.4 \pm 0.65$         | $42.1 \pm 1.96$         |
| Free $T$ , pmol/liter    | $4.31 \pm 0.09$   | $0.93 \pm 0.06^*$     | $2.13 \pm 0.08^{**}$    | $1.91 \pm 0.05^{**}$    |
| %                        | $9.4 \pm 1.3$     | $2.29 \pm 0.1^*$      | $4.12 \pm 0.09^{**}$    | $3.43 \pm 0.2^{**}$     |
|                          | 100               | $21.6 \pm 1.39$       | $49.4 \pm 1.86$         | $44.3 \pm 1.16$         |
|                          |                   | $24.3 \pm 1.06$       | $43.8 \pm 0.95$         | $36.4 \pm 2.12$         |

than that in birds of groups 2 and 1 (by 14 and 2.8 times, respectively). The shape of thyrocytes remained unchanged in chickens of the PPP group. These cells remained cubical. However, the height of thyrocytes decreased. The nuclear-cytoplasmic ratio in these birds increased less significantly than in group 2 chickens. Similar, but less significant changes were found in old hypophysectomized birds of the PPH group (Table 1).

The concentration of TTH,  $T_3$ ,  $T_4$ , and free thyroxin in PPP-receiving chickens was shown to increase more significantly than in old hens (Table 2).

Our results indicate that APP and PPP prevent the signs of hypothyroidism and normalize the structure and function of the thyroid gland in chickens and old birds. Peptides Lys-Glu-Asp-Gly and Ala-Glu-Asp-Gly have a similar effect on morphological characteristics and hormonal function of the thyroid gland, which is related to the same sequence of 3 amino acids (Glu-Asp-Gly) in both tetrapeptides.

Peptide treatment is followed by an increase in the concentration of TTH in hypophysectomized birds, since pituitary hormones (e.g., TTH) can be synthesized in various tissue and cells of the APUD system, thymus, and lymphocytes [9-11].

Our results indicate that hypophysectomy in birds during the early stage of ontogeny is followed by more pronounced changes in the endocrine status compared to those in old hens. Peptide treatment was shown to

prevent abnormalities in the endocrine system. It should be emphasized that the degree of hormonal recovery in old hens was lower than in chickens. Undoubtedly, the observed differences are associated with less significant compensatory reaction in old animals [5,7-9].

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