Histogical structure of the thyroid gland in rabbits with different types of autonomous tonus

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The research was aimed at investigating the influence of the aggregated tonus of sympathetic and parasympathetic centers on the rabbit’s thyroid gland morphology. For this purpose, the rabbit males (Oryctolagus cuniculus (Linnaeus, 1758)) of Blanc de Termond breed at the age of four months, passed an electrocardiographic and variation pulsometry study. According to the results, the animals were divided into three groups: rabbits with sympathicotonia (18 animals), rabbits with normotonia (5 animals) and rabbits with parasympathicotonia (3 animals). After euthanasia, the fragments of the thyroid gland were taken out, fixed in Bouin’s solution and embedded in paraffin blocks. Histological sections were prepared from the paraffin blocks and later stained with hematoxylin and eosin, as well as Gabu-Diban’s aldehyde fuchsin and Heidenhain’s azan. Morphometric study of follicles and cells of the gland was performed using these histopreparations. Based on the obtained data, the indices of gland activity were calculated, i.e. Brown index and the follicle-colloid index. At the same time, it was established that morphofunctional parameters of the thyroid gland depend on the typological features of the autonomous tonus. This is reflected in the thickness of the capsule, the amount of adipose tissue, the size and density of the follicles’ placement, the height of the thyrocytes and the ratio between the areas of their nuclei and the cytoplasm. Rabbits with parasympathicotonia account for the higher values of half (7 from 14) of the studied parameters, while their combination suggests the functional activity of the thyroid gland of the animals in this group is higher compared to the other groups of rabbits. Also, rabbits with parasympathicotonia have a larger body weight. For normotonic and sympathicotonic rabbits, the functional activity of the thyroid gland as well as the body weight is lower and approximately of the same intensity. The existence of correlation between the separate indicators was investigated, the character of which is also related to the type of the autonomous tonus.

Key words: parasympathicotonic rabbits, normotonic rabbits, sympathicotonic rabbits, thyrocytes.
Introduction

Thyroid gland belongs to the hypothalamic-pituitary-thyroid system (Ortega-Carvalho et al., 2016). In rabbits, it consists of two parts connected by a thin isthmus and located on the larynx and trachea within the range from the anterior horn of the thyroid cartilage to the 9th tracheal cartilaginous ring (Zhedenov, 1987). The hormones the thyroid gland produces affect the growth of the organism, and the development of sexual and nervous system and are also involved in the metabolism (Jost, 1954; Krassas et al., 2010; Forhead & Fowden, 2014; Coll, 2014; Mullur et al., 2014). Therefore, the structure of the gland of animals in general and rabbits in particular, was subjected to a comprehensive study for quite a long time. The study of the functional states of the organism (Sugiyama & Yagizawa, 1951; Balasundaratn & Mookkappan, 2000; Parchani et al., 2012; Onwuaso et al., 2015; Johansson et al., 2015) and pathology (Alcaraz et al., 2009; Borges et al., 2014). In addition, a number of scholars (Sugiyama, 1954; Abdelatif & Saeed, 2009; Al-Mustawfi et al., 2011; Bessalova, 2012; El-Desouki et al., 2014; Gachkar et al., 2019) conducted experimental research studying the relationship between the anatomical and physiological parameters of the gland and certain functional states of the organism. Despite a large number of studies, we have not found any data that would prove the effect of different tonus of the autonomic nervous system on the morphology of the thyroid gland of rabbits. At the same time, a similar effect was established on the structure of the adrenal gland (Zakrevska & Tybinka, 2019). Studying this issue was the aim of our research.

Materials and methods

Animals: twenty six clinically healthy rabbit-males (Oryctolagus cuniculus (Linnaeus, 1758)) of Blanc de Termond breed, four months of age. The device MK-15,2-TN20 was used to determine the weight of animals. Euthanasia was performed by inhalation overdose of chloroform.

For all animals, an electrocardiographic study was conducted. For this purpose, a rheograph (model P4-02) with an electrocardiographic channel and a recording device (model H338-6P) was used. The velocity of the tape when recording the cardiac signal was 250 mm/s. The received electrocardiograms were processed using a method of variation pulsmetry with a study of at least 100 R-R intervals (Baevskij et al., 1984). Based on the obtained data, the animals were divided into three groups:

1) sympathetic tonic rabbits (ST) – 18 animals;
2) normotonic rabbits (NT) – 5 animals;
3) parasympathetic-tonic rabbits (PS) – 3 animals. In ST rabbits, the tonus of sympathetic autonomous centers clearly dominated over that of the parasympathetic ones. In NT rabbits, the tonus of the sympathetic and parasympathetic centers is balanced. Accordingly, in the PS rabbits, the tonus of parasympathetic centers is dominant. When carrying out a statistical analysis of the research results, the group of sympathicotonic rabbits was taken as the basis for comparing the performance of the other two groups.

For histological study, fragments of the thyroid gland were taken out of each rabbit and fixed in Bouin’s solution. After 24 hours, the pieces of histological material were conducted through an upward row of alcohols, each of which was kept for 24 hours and embedded in paraffin blocks afterwards. Cuts with the thickness of 7 µm were prepared from the obtained blocks on a slide glass and dried, then stained with Mayer’s aldehyde fuchsin (Falin, 1961). The histological preparations were examined under a Leica DM-2500 microscope equipped with a Leica DFC450C camera. Morphometry was performed using the Aperio Image Scope software. In this case, the following indicators were determined: the follicle area, the area of follicle lumen, the epithelium height, the thyrocyte area, the thyrocyte nucleus area.

Based on these indicators, the following parameters were calculated: 1) diameter of the follicle (d = 2(√(S/π)), where: d – the follicle diameter, S – the follicle area, π – the mathematical constant = 3,14); 2) number of follicles per 1000 mm²; 3) area of the follicle epithelium (the difference between the area of the follicle and its lumen); 4) area of the thyrocyte cytoplasm (the difference between the area of the cell and its core); 5) nuclear-cytoplasmic ratio or NC ratio (nucleus area/cytoplasm area); 6) Brown index (follicle diameter/2*(follicle epithelium height)); 7) follicle-colloid index (epithelium area/colloid area) (Hmelnitsky, 1998; Bessalova, 2012).

Statistical analysis of the research results as well as correlation analysis were carried out using the StatPlus2008 software. In this case, the following parameters were calculated: x – sample mean, SE – standard error of the mean, r – correlation by Pearson. Student’s t test was used to establish the validity of the differences between the indices of separate groups of rabbits. In the table, this validity was represented as follows: * – P < 0.05; ** – P < 0.01; *** – P < 0.001.
During the research, the ethical standards for animals used for experimental purposes (Strasbourg, 1986, Kyiv, 2001) were fully observed and the methodology for conducting research was approved by the Ethics Commission of the Stepan Gzhaltytsky National University of Veterinary Medicine and Biotechnologies Lviv (2016).

**Results**

The thyroid gland we studied had a pink color with a pale brown shade and was typically located (Fig. 1). The thyroid gland capsule consists of three layers: external or serous one, medium - fatty vessels and nerves and internal or fibrous one. The inner layer (own capsule of the gland) penetrates into the thickness of the gland and forms a connective tissue frame for follicles, which is mainly constructed of collagen fibers (Fig. 2).

![Fig 1. Topography of rabbit’s thyroid gland: 1 – thyroid gland, 2 – thyroid cartilage, 3 – ring-shaped muscle, 4 – trachea, 5 – submandibular salivary gland. Gross specimen](image)

![Fig 2. Fragment of the ST rabbit’s thyroid gland: 1 – capsule, 2 – fat layer, 3 – vessels, 4 – connective tissue hives, 5 – lumen of the follicle with colloid. Gabu-Dibhan’s aldehyde fuchsin 10x10](image)

The thickness of the capsule varies greatly even within the same gland, due to the uneven location of the fatty layer (Fig. 3, 4).

![Fig 3. Fragment of thyroid gland of NT rabbit: capsule (arrows). Heidenhain’s azan 10x10](image)

![Fig 4. Fragment of thyroid gland of NT rabbit: capsule (arrows). Heidenhain’s azan 10x10](image)

![Fig 5. Fragment of the thyroid gland of PS rabbit: 1 – fatty tissue, 2 – groups of follicles, 3 – vessel. Heidenhain’s azan 10x10](image)

It is best of all expressed in PS rabbits. The indicator of thickness varies from 65.88 μm to 478.6 μm and the capsule itself is the thickest among the studied groups (Table 1). The thinnest capsule was found in ST rabbits (300.05 ± 35 μm). The difference between these groups
amounts to 82.65 μm.

In addition to the capsule, fatty tissue is also represented in the gland parenchyma, occupying large areas and surrounding the groups of follicles (Fig. 5).

Table 1
Body weight and morphometric indices of the thyroid gland of rabbits in different groups (x ± SE)

| Indicator                          | Sympathicotonic rabbits | Normotonic rabbits   | Parasympathicotonic rabbits |
|------------------------------------|-------------------------|----------------------|-----------------------------|
| Weight of rabbit, kg               | 3.66 ± 0.09             | 3.67 ± 0.24          | 3.92 ± 0.43                 |
| Capsule thickness, μm              | 300.05 ± 35.00          | 309.04 ± 27.24       | 382.70 ± 68.11              |
| Follicle area, μm                  | 6108.46 ± 441.16        | 4376.48 ± 792.62     | 2519.62 ± 703.02            |
| Follicle lumen area, μm²           | 4506.71 ± 385.56        | 2719.77 ± 487.79     | 1380.43 ± 571.59            |
| Diameter of follicle, μm           | 84.37 ± 3.10            | 95.86 ± 25.56        | 54.22 ± 7.46                |
| Number of follicles per 1000 μm²  | 180.14 ± 23.50          | 266.50 ± 72.66       | 260.84 ± 76.61              |
| Epithelium area, μm²               | 1601.76 ± 78.57         | 1656.71 ± 310.63     | 1139.20 ± 138.62            |
| Epithelium height, μm              | 6.38 ± 0.21             | 7.54 ± 0.65*         | 7.61 ± 0.65*                |
| Thyrocyte area, μm²                | 60.40 ± 2.43            | 61.48 ± 3.00         | 89.56 ± 12.26               |
| Nucleus area, μm²                  | 23.48 ± 0.81            | 21.68 ± 1.40         | 25.57 ± 0.58                |
| Cytoplasm area, μm²                | 36.69 ± 1.76            | 39.80 ± 2.22         | 64.00 ± 12.03               |
| Nuclear-cytoplasmic ratio         | 0.70 ± 0.02             | 0.58 ± 0.03***       | 0.47 ± 0.11***              |
| Brown index                        | 12.85 ± 1.04            | 7.10 ± 0.81**        | 7.28 ± 1.90                 |
| Follicle-colloid index             | 0.39 ± 0.02             | 0.61 ± 0.03***       | 1.14 ± 0.44**               |

Note: statistically reliable difference compared with the group of sympathicotonic rabbits (* – P < 0.05; ** – P < 0.01; *** – P < 0.001).

Follicles of the gland are of a various size and a round-oval shape mostly. However, the follicles of irregular shape systematically occur (Fig. 6). Apparent regularities of this phenomenon were not established. Nevertheless, when measuring the follicles area, significantly different results were received in experimental groups. Thus, the largest follicles were found in ST rabbits. Their area exceeds the same parameter for NT animals by 1731.98 μm². The area of follicles was the smallest in the group of PS rabbits. In fact, it was 3588.84 μm² smaller than in ST rabbits.

Fig 6. Follicles of different size and shape of NT rabbit. Hematoxylin and eosin 10x40

Difference between the areas of the follicle and its lumen allowed to calculate the area of the follicle epithelial layer. In NT and ST rabbits, it is almost identical and exceeds the PS group by 517.51 μm² and 462.56 μm² respectively.

An increase in the area of the epithelium occurs due to an increase in the height of its cells. Thus, in ST rabbits, the height of epithelial cells is the lowest and is 1.16 μm less than in NT rabbits (P < 0.05) and 1.23 μm less than in PS rabbits (P < 0.05) (Fig. 7, 8).

Fig 7. Follicles of ST rabbit: 1 – cells of flat form (tyrocytes), 2 – lumen of follicle with colloid. Hematoxylin and eosin 10x100

Studying the diameter of the follicles, it was found that its highest values correspond to NT rabbits and the lowest to PS rabbits. However, these figures are not reliable.

Compared to the other animal groups, the follicles of NT rabbits are distinguished not only by their larger size, but also by their denser location, which means their quantity per 1000 μm² of gland is the largest. This occurs due to a smaller amount of interfollicular connective tissue, in particular fat (Fig. 5, 9). In ST rabbits, this area includes 86.4 follicles less. Despite such a significant difference between the indicators, it is still not reliable, due to the uneven location of the follicles in the gland tissue, where the areas with high saturation of follicles are combined...
with the sites with significant content of stroma (Figures 9, 10).

**Fig 8.** Follicles of NT rabbit: 1 – cells of cubic and cylindrical form (tyrocytes), 2 – lumen of follicle with colloid. Hematoxylin and eosin 10x100

**Fig 9.** Density of the location of ST rabbit’s follicles: 1 – adipocytes, 2 – vessel. Hematoxylin and eosin 10x40

**Fig 10.** Density of the location of ST rabbit’s follicles. Hematoxylin and eosin 10x40

The specified pattern is the most characteristic for PS and NT animals. According to this indicator, the PS rabbits also significantly dominate ST animals, namely by 80.7 follicles.

In the group of NT rabbits, a weak direct correlation \( r = 0.32 \) was found between the number of follicles and the weight of rabbits. In two other groups, the correlation is inverse: for PS rabbits, \( r = -0.21 \), and for ST rabbits, \( r = -0.07 \).

Between the follicles filled with colloid, clusters of tyrocytes in the form of interfollicular islets sometimes occur (Fig. 11, 12). This process is characteristic for all groups of animals. It is of no functional significance (Kachalka, 1986) and therefore its research was not carried out.

**Fig 11.** Accumulation of tyrocytes among the follicles of PS rabbit (arrows). Hematoxylin and eosin 10x40

**Fig 12.** Accumulation of tyrocytes among the follicles of ST rabbits. Hematoxylin and eosin 10x100

Each follicle is covered with a single-layered cubic epithelium, which, depending on the functional state, can change its shape to a cylindrical or flat (Fig. 7, 8). The epithelium consists of two types of cells: tyrocytes and parafollicular C-cells (Fernández-Santos et al., 2012). In our research, only the first type of cells was studied. At the same time, the thyrocytes with the largest area were detected in PS rabbits and they exceed ST animals by 29.16 \( \mu m^2 \). For NT rabbits, intermediate values are observed.

Analyzing the structural parts of thyrocytes, it was
found that PS rabbits had the highest values of both nucleus and cytoplasm area among the experimental groups of animals. In NT rabbits, the smallest nucleus area is combined with the average cytoplasm area, while in ST animals, on the contrary, the average nucleus area corresponds to the minimal cytoplasm area. However, the difference between these indicators in all groups of animals is not reliable.

The study of the morphofunctional state of cells was performed on the basis of three indices: nuclear-cytoplasmic ratio (NC ratio), Brown index and follicle-colloid index.

So, the NC ratio is the largest in ST rabbits. NT rabbits occupy the second place with the ratio lower by 0.12 units (P < 0.001). PS rabbits have the smallest NC ratio, lower by 0.23 units (P < 0.001). These difference of NC ratio occurs more due to the difference in the cytoplasm area, since the area of the nuclei varies by no more than 3.89.

ST rabbits are also characterized by the highest values of the Brown index, exceeding PS rabbits by 5.57 units and NT rabbits by 5.75 units (P < 0.001).

An entirely different type of autonomous tonus manifests itself in the values of the follicle-colloid index. Its lowest values correspond to ST rabbits. Accordingly, the NT animals dominate by 0.22 units and the PS ones – by 0.75 units.

Correlation between the mentioned indices and the body weight of animals was also investigated. It was established that the weight of PS and ST rabbits had the inverse correlation with the Brown index, i.e. \( r = -0.67 \) and \( r = -0.22 \) respectively and the inverse correlation with the follicle-colloid index, i.e. \( r = -0.74 \) and \( r = -0.22 \) respectively. In NT animals, the Brown index has a strong inverse correlation with body weight (\( r = -0.87 \)), while the follicle-colloid index has a weak direct correlation (\( r = 0.11 \)).

At the same time, it should be noted that all the represented correlations are not reliable.

**Discussion**

The structure of the thyroid gland capsule of rabbits is similar to the same structure in chickens described by Balasundaratn and Mookkappan, 2000. In this case, the outer layer of the capsule is a continuation of the pretracheal plate of the cervical fascia. In both species, the majority of connective tissue fibers of the capsule is collagenous, which was also found in African reed rats (Igbokwe, 2010). The uneven location of adipose tissue in different parts of the capsule occurs partly due to its protective function and, in our opinion, is associated with the peculiarities of the gland topography with regard to the other organs (larynx, trachea, muscles and skin). To a certain extent, typological peculiarities of the autonomous tonus are reflected both in the amount of adipose tissue and in the thickness of the gland capsule.

Different size of follicles is related to their functional state. Follicles of a larger diameter are located mainly in the central part of the gland and characterized by a reduced functional activity. The periphery is more functionally filled with active follicles of the smaller size. A similar situation was also observed in mice, humans (Lee et al., 2016) and birds (Moghanlo & Mohammadpour, 2018). The reason for such uneven location of the follicles is the development process of the gland that begins from its central part and is shown on the example of laboratory rats (Vojtkevich & Zenerov, 1968). The number of follicles increases until the moment of puberty due to the division of other follicles (Kachalka, 1986). At the same time, it was found that intrafollicular islets, which were also detected in our studies are not the embryos of the new follicles, but the cuttings of their tops. However, Igbokwe, 2010 describes the opposite principle of the location of follicles in the thyroid gland of wild rats, in which small follicles are located in the central part of the gland, while large and medium ones – on its periphery. Such differences may occur due to species-specific and environmental factors.

The irregularity of shape in some follicles may be explained by the fact that secretion in the adjacent follicles does not occur simultaneously, since they are at different stages of their functional cycle. Another reason for this phenomenon is probably the division of thyrocytes, resulting in the formation of an epithelium fold, which immerses into the follicle lumen in the form of a papilla (Kachalka, 1986). Follicles of an irregular shape are also a characteristic feature of the thyroid gland of rats (Onwuaos et al., 2015).

The use of indices allows to characterize the functional features of the thyroid gland based on its morphological parameters (Davis & Davis, 1954; Firdous & Karalathukaran, 2013). Thus, Brown index and follicle-colloid index characterize the activity of the gland based on its ability to accumulate colloid and synthesize iodine-containing hormones. At the same time, the decrease of the first index and the increase of the second one, observed in PS rabbits, indicate an increase in the hormonal activity of the gland. The opposite option (increase of the first index and decrease of the second one), indicates a decrease in the hormonal activity of the gland passing on the background of high colloid secretion. To a different extent, this phenomenon is observed in NT and ST rabbits. Similar signs reveal themselves during the hypofunction of the gland, especially in the process of aging (Lee et al., 2016).

An increase in the amount of colloid caused by the decreased follicle activity leads to stretching and thinness of its walls. As a result, the shape of epithelial cells changes from cubic to flat, which corresponds to the data of Moghanlo and Mohammadpour, 2018. Also, the location of microvilli on the apical surface of the follicles is changing (Parchami & Fatahian Dehkordi, 2012). Among the animals we studied, the sites with expanded follicles (Fig. 13) were detected in two NT rabbits (40 %) and six ST rabbits (33 %). In PS rabbits, such follicles were not detected. Their follicles were of a small size with a cylindrical epithelium. (Fig. 14).

Functional activity of the follicles can be evaluated based on the color of colloid inside them. According to Moghanlo and Mohammadpour, 2018, smaller, more active follicles contain a darker colloid, while larger and
less active follicles contain a lighter colloid. The indicated pattern was also confirmed during our research.

**Fig 13.** Follicles of the thyroid gland of ST rabbit. Hematoxylin and eosin 10x40

**Fig 14.** Follicles of the thyroid gland of PS rabbit. Hematoxylin and eosin 10x40

Morphofunctional parameters of thyroid gland of rabbits are characterized by different levels of correlation with the body weight of animals. To a certain extent, typological peculiarities of the autonomous tonus regulate this process. Similar correlations were found in birds, however, the features of autonomous regulation weren’t taken into account (Firdous et al., 2012).

**Conclusion**

A correlation between the structure of the thyroid gland and the typological features of the autonomous tonus was established in the rabbits (*Oryctolagus cuniculus* (Linnaeus, 1758)). Considering all the morphometric indices and ratios between them, the PS rabbits are characterized by a functionally more active thyroid gland, which is also reflected in the higher values of body weight. Morphometric indices of thyroid gland of NT and ST rabbits indicate that the functional activity of the gland in these groups is approximately equal, but lower compared to the PS animals. A similar situation refers to the body weight. Considering seven out of fourteen indicators, PS rabbits outweigh other groups of animals.

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