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Conflict of interest. The authors declare that they have no conflict of interest.

ORCID: Bieliaiev I.S. <https://orcid.org/0009-0000-2253-3041>, Ulanovskaya-Tsyba N.A. <https://orcid.org/0000-0002-5093-5686>, Zviahol'ska I.M. <https://orcid.org/0000-0001-7531-3966>, Kononenko S.V. <https://orcid.org/0000-0002-9423-5104>, Tymoshenko Yu.V. <https://orcid.org/0000-0003-4023-5552>, Kokovska O.V. <https://orcid.org/0000-0002-8277-6229>, Ishchenko V.I. <https://orcid.org/0000-0003-3513-2110>.

Article received: 22.09.2025

DOI 10.26724/2079-8334-2026-3-97-224-230

UDC 616.316.1-092-071

**Hryn V.H., Tikhonova O.A., Piliuhin A.V., Tarasenko Ya.A., Fedorchenko I.L.,
Hryn M.V., Shevchenko O.M.
Poltava State Medical University, Poltava, Ukraine**

SEROUS DEMILUNES OF GIANNUZZI AND MORPHOFUNCTIONAL CHARACTERISTICS OF THE MAJOR SALIVARY GLANDS

e-mail: fedorchenkoigor@ukr.net

The salivary glands are key exocrine organs that maintain oral homeostasis, facilitate primary digestion, and provide immune defense. This review article summarizes historical and contemporary data on the morphology of the major salivary glands in humans and rodents, including the parotid, submandibular, and sublingual glands. Particular attention is focused on the serous demilunes of Giannuzzi, their morphological organization, cellular specialization, and secretory function. The study analyzes species-specific differences in the acini and ductal system, as well as the mechanisms of secretion for enzymes, mucins, and growth factors. The significance of historical research methods for the modern understanding of the morphofunctional organization of the glands and their clinical relevance is discussed.

Key words: salivary glands, serous demilunes of Giannuzzi, acini, ductal system, parotid gland, submandibular gland, sublingual gland.

**Гринь В.Г., Тихонова О.О., Пілюгін А.В., Тарасенко Я.А., Федорченко І.Л.,
Гринь М.В., Шевченко О.М.
СЕРОЗНІ «ПІВМІСЯЦІ» ДЖІАННУЦЦІ ТА МОРФОФУНКЦІОНАЛЬНА
ХАРАКТЕРИСТИКА ВЕЛИКИХ СЛИННИХ ЗАЛОЗ**

Слинні залози є ключовими екзокринними органами, що забезпечують ротовий гомеостаз, первинне травлення та імунний захист. У цій оглядовій статті узагальнено історичні та сучасні дані про морфологію великих слинних залоз людини та гризунів, включаючи привушну, піднижньощелепну та під'язикову залози. Особлива увага приділена серозним «півмісяцям» Джіаннуцці, їхній морфологічній організації, клітинній спеціалізації та секреторній функції. Проаналізовано видові відмінності ацинусів та протокової системи, механізми секреції ферментів, муцину та факторів росту. Обговорено значення історичних методів дослідження для сучасного розуміння морфофункціональної організації залоз та їх клінічної значущості.

Ключові слова: слинні залози, серозні «півмісяці» Джіаннуцці, ацинуси, протокова система, привушна залоза, піднижньощелепна залоза, під'язикова залоза.

The study is a fragment of the research project "Systemic analysis of the morphological and functional state of various internal organs and body systems under the influence of irritants and mechanical injuries", state registration No. 0125U002785.

The salivary glands, as an anatomical and physiological structure, are key components of human oral homeostasis, as well as the digestive and immune systems. They facilitate the secretion of saliva, which is rich in enzymes, mucins, immunoglobulins, electrolytes, and biologically active substances. These components participate in

the initial processing of food, regulation of the microbiota, lubrication of the mucous membranes, and provision of antimicrobial defense [1, 5, 9]. These exocrine glands produce 0.5–1.5 litres of saliva every day, which, in turn, helps to facilitate chewing, swallowing and speech, lubricates the mucous membrane of the mouth and provides an aqueous

environment for taste perception. The salivary glands are also involved in the digestion of triglycerides and starches by secreting lipases and amylases. The salivary glands are also known to play a protective role against infections thanks to their numerous organic components – the secretory fraction (glycoprotein, lysozymes, autolysin, lactoferrin). The first forms a complex with immunoglobulin A (IgA) to protect against viruses and bacteria; the second causes agglutination of bacteria; the third functions to destroy bacterial cell walls; and the fourth binds iron. The salivary glands, as an anatomical and physiological structure, are key components of human oral homeostasis, as well as the digestive and immune systems. They facilitate the secretion of saliva, which is rich in enzymes, mucins, immunoglobulins, electrolytes and biologically active substances. These components play a role in the initial processing of food, the regulation of the microbiota, the lubrication of the mucous membranes and the provision of antimicrobial defence [8].

It is well known that the salivary glands act as reservoirs for infections, and the SARS-CoV-2 virus is no exception. The mechanisms underpinning infection and replication in the salivary glands remain poorly understood due to the lack of suitable *in vitro* models. A study by Tanaka J [25] and colleagues showed that human-induced salivary glands (hiSG), derived from human-induced pluripotent stem cells, can be infected with SARS-CoV-2. HiSG exhibit properties similar to those of embryonic salivary glands and are a valuable tool for the functional analysis of genes during development. The authors have established that hiSG may be a promising model for investigating the role of the salivary glands as a viral reservoir.

Given their complex structure, functional cellular specialization, and regulatory interaction with the nervous system, salivary glands have long remained a subject of close attention for morphologists, histophysiologicals, biochemists, dentists, oncologists, and clinicians.

The study of salivary gland structure is particularly important in the context of contemporary interdisciplinary research that integrates morphology, molecular biology, and clinical practice. Morphofunctional disorders of the salivary glands are of significant clinical importance in pathologies such as Sjögren's syndrome, sialadenitis, benign and malignant tumors, metabolic and autoimmune lesions, and post-radiation changes resulting from the treatment of head and neck tumors [1, 2, 6-9, 13, 15, 17, 18, 20, 23].

With regard to malignant tumours, it is worth noting that regenerative medicine techniques are now in use which enable the epithelium of the salivary gland to be replaced or regenerated and its secretory function to be restored. It was in rodents that early strategies for adult stem cell transplantation were tested, demonstrating improved clinical outcomes in

radiation-induced xerostomia. Animal models, particularly those involving rodents and pigs, are essential components of these morphological and histological studies [3, 4, 10-12, 14-16, 19-22, 27].

Animal models have served as a basis for a number of biochemical, morphological and histological studies; in particular, there are well-known studies investigating the effects of low doses of aluminium chloride on the oxidative biochemistry, proteomic profile and morphology of the major salivary glands, carried out on male rats. Long-term cultures of mouse and human salivary gland organoids have been established, enabling the creation of tumour cultures from both benign and malignant types of salivary gland tumours, in which tumour-specific gene signatures are well preserved [23, 28, 30].

The use of a mouse submandibular gland model provided the basis for establishing the role of the Sox9-mediated pathway as a promising candidate for the regeneration of damaged salivary glands, whilst a transgenic mouse model of Apert syndrome demonstrated that the Ser252Trp substitution in mouse FGFR2 led to hyperplasia of the submandibular gland parenchyma during development [26, 29].

In this regard, there is a growing need for a fundamental understanding of the structure, cellular organization, and secretory mechanisms of these glands. Historical and scientific data play a crucial role in this process, allowing researchers to trace the origins of modern concepts and critically reflect on the methodological evolution in the study of glandular organs.

The contribution of 19th-century researchers holds particular scientific value, as the foundations of modern functional histology were laid during this period through the integration of morphological analysis with elements of experimental physiology. In this context, the figure of Giuseppe Ortonzo Giannuzzi deserves special attention [8]. His research on the morphology of serous cells, secretion dynamics, and the microscopic organization of the salivary glands became milestones in the development of histology as a science. Specifically, the morphofunctional changes in the acinar apparatus described by him, including the discovery of the serous "demilunes," became the basis for decades of subsequent morphological, biochemical, and clinical research [8, 24].

In the present day, as the study of cellular secretory mechanisms is complemented by knowledge from molecular biology, transmission electron microscopy, and 3D visualization, revisiting classical studies allows for a better understanding of the genesis of concepts and methods currently in use. The analysis of Giannuzzi's scientific heritage is not only a tribute to history but also an important tool in the development of modern medical science, as it enables the integration of morphological knowledge

into a broader biomedical context. Thus, the relevance of this topic is determined not only by the scientific and historical significance of the scientist's research but also by its direct influence on the development of medical morphology, clinical anatomy, histopathology, and functional diagnostics of the salivary glands in the 21st century.

The purpose of the study was to evaluate the morphological organization of the major salivary glands and the historical and scientific significance of the discovery of the serous demilunes of Giannuzzi.

Materials and methods. Bibliographic Basis of the Review. The study utilized publications from international scientific databases such as PubMed, Scopus, Web of Science, SpringerLink, and Google Scholar, covering the anatomy, histology, and functional organization of the major salivary glands in humans and rodents. Open electronic archives of historical scientific works and libraries were also consulted. Additionally, classical works from the 19th and 20th centuries, which established the morphological concepts and interpretations of the serous demilunes of Giannuzzi, were included.

Source Selection Criteria. The analysis included: articles in peer-reviewed journals (experimental, clinical, and morphological studies); monographs and textbooks on anatomy, histology, and embryology; and historical primary sources of scientific and practical significance. Materials lacking scientific peer review (popular reviews, incomplete conference abstracts, and secondary translations without references) were excluded.

Search Strategy. The search was conducted using the following keywords: "salivary glands", "parotid", "submandibular", "sublingual", "histology", "serous demilunes", "Giannuzzi". The timeframe spanned from classical 19th-century descriptions to contemporary research through the 2020s.

Analysis Methodology. A comparative analysis of the morphological characteristics of human and rodent salivary glands was performed. Data regarding the structure of acinar and ductal systems were systematized. A historical-scientific approach was applied to assess the contribution of Giannuzzi and his followers to the development of knowledge concerning serous demilunes. A critical review of modern research, including immunohistochemical and morphometric studies clarifying the role of the glands in physiology and pathology, was also conducted.

Analytical Critique of Sources. A critical evaluation of the reliability and authority of the utilized publications was performed, taking into account their chronological, contextual, and linguistic characteristics. A careful distinction was made between primary sources (original works) and secondary sources (modern interpretations and historical reviews).

Results of the study and their discussion.

Major Salivary Glands: Morphology and Histological Features. Salivary glands are exocrine organs that synthesize, modify, and secrete saliva into the oral cavity. They are classified into major glands (parotid, submandibular, and sublingual) and minor glands, which are distributed throughout the mucous membrane of the upper digestive tract and the oral cavity. Overall, the major salivary glands produce approximately 92–95 % of the total salivary volume, while the minor glands contribute the remainder, ensuring continuous lubrication of the oral mucosa.

Saliva performs numerous physiological functions. It facilitates mastication, swallowing, and speech activity, moistens the oral mucosa, and creates an environment for taste perception. Enzymes contained in saliva, such as amylase and lipase, participate in the initial phase of carbohydrate and lipid digestion. Furthermore, saliva serves a protective function due to its organic components: the secretory fragment of IgA forms a complex for protection against viruses and bacteria, lysozymes promote bacterial agglutination, and lactoferrin binds iron, thereby limiting bacterial growth. Ionic compounds, particularly bicarbonates, buffer the acids produced by bacteria, protecting the oral cavity and the esophagus from the effects of gastric juice. Thus, saliva prevents the development of chronic mucosal infections and dental caries.

The macroscopic structure of the major salivary glands in humans and white rats exhibits similarities, yet significant differences exist. In humans, the parotid gland is situated within a triangle bounded by the zygomatic arch, the masseter muscle, and the sternocleidomastoid muscle. In rodents, however, it is localized behind the auricle and is partially covered by subcutaneous adipose tissue. The submandibular and sublingual glands in humans are positioned below and above the mylohyoid muscle, respectively, whereas in rodents, they form a tight complex in the anterior part of the neck. The main excretory ducts of these glands open into the oral cavity, ensuring the discharge of secretion, and they exhibit morphological variations across different species [27].

As observed, the major salivary glands share common anatomical and histological features, although each gland differs in its topography and the specifics of its ductal system. Externally, the glands are covered by a thin fibrous capsule, from which connective tissue septa extend deep into the gland, forming a stromal framework that supports the acini and ducts. Adipose depots of both white and brown types, as well as lymph nodes, are often located adjacent to the glands, emphasizing the anatomical integration of the salivary glands with the systems of immune defense and metabolism.

The acini, the primary secretory units of the gland, are composed of serous, mucous, or mixed cells. Serous cells are characterized by a high content

of rough endoplasmic reticulum, Golgi complexes, and numerous mitochondria, which facilitate active protein secretion. Mucinous acini form secretory granules that often appear as clear vacuoles within the cytoplasm. Myoepithelial cells surround the acini and intercalated ducts, promoting the active excretion of saliva into the ductal system.

The ductal system of the major salivary glands includes several types of ducts: intercalated, convoluted (granular), and intralobular (striated) ducts. The ductal system extends into the interlobular space and consists of excretory ducts that merge to form the main excretory duct.

Intercalated ducts are formed by low cuboidal epithelium and provide the transition from the acini to the more complex secretory portion of the ducts. Intralobular ducts are distinguished by a high columnar epithelium with basal striations, reflecting the active transport of sodium and potassium ions. Excretory ducts are lined with columnar epithelium and terminate by opening into the oral cavity, often exhibiting an increased diameter and expansion at the terminal segment [27].

The detailed microscopic structure of rat salivary glands differs from that of humans. In rodents, the submandibular glands contain convoluted (granular) ducts that secrete numerous growth factors and hormone-like molecules, such as EGF, NGF, BDNF, HGF, IGF-I, TGF- α , and TGF- β . These factors not only act locally on the mucous membrane but can also enter the bloodstream and affect distant organs, performing endocrine and paracrine functions. In humans, granular ducts are absent; however, the secretion of growth factors occurs through serous acini and intralobular ducts, which supports protective and regenerative processes in the mucous membranes and digestive tract organs [2].

The secretory acini are composed of serous and mucous cells, where serous granules contain large amounts of water and ions, while mucous cells contain mucin and glycoconjugates (glycoproteins, glycolipids, and proteoglycans). In mixed acini, serous demilunes ("Giannuzzi demilunes") surround the mucous cells, ensuring optimal mixed secretion. Microstructural features include well-developed intercalated and intralobular ducts, which participate in the transport of electrolytes, water, and immunoglobulins, and also contain potential progenitor cells capable of regenerating acini and ducts [2].

The submandibular gland is the largest, featuring an extensive system of intralobular and convoluted (granular) ducts, with pronounced sexual dimorphism during the postnatal period. In males, the ducts are wider and contain a greater number of secretory granules, which is attributed to the hormonal dependence of the cells.

The sublingual gland is smaller, consisting predominantly of mucous acini and a simpler ductal

system; its ducts often run parallel to the submandibular duct but open separately into the oral cavity.

The parotid gland consists of serous acini, which are histologically similar to the exocrine portion of the pancreas, and a complex system of interlobular ducts. The intercalated ducts of the parotid gland play a vital role in the secretion of electrolytes into the saliva.

The anatomical and histological organization of the major salivary glands is closely linked to their function: the acini produce the primary secretion, while the ductal system modulates the salivary composition, including the electrolyte balance. Contemporary studies emphasize the significance of ultrastructural changes in acinar and ductal cells across different developmental periods, as well as the influence of hormonal factors on glandular morphogenesis. These data possess applied significance for physiology, pathophysiology, and the development of experimental models for salivary gland diseases [8].

Blood supply to the major glands is provided by branches of the external carotid artery: the parotid gland via the superficial temporal and zygomatic arteries; the submandibular gland via the facial artery; and the sublingual gland via the sublingual and submental arteries. Venous drainage occurs through the corresponding veins, forming the retromandibular and external jugular systems. Innervation includes both parasympathetic and sympathetic components. Parasympathetic fibers stimulate serous secretion and ion transport, while sympathetic fibers regulate protein secretion and blood flow. Minor salivary glands secrete saliva autonomously, independent of neural control.

Functional disorders of the salivary glands, such as xerostomia or sialorrhea, are often associated with systemic diseases (endocrine disorders, diabetes mellitus, atherosclerosis), neurological impairments, or drug therapy. Common pathologies include sialadenitis, sialolithiasis, and the formation of mucocoeles and ranulas. Sialoliths (salivary stones) most frequently form within the excretory ducts, particularly in Wharton's and Stensen's ducts, causing pain, swelling, and inflammation. Minor mucocoeles occur due to traumatic injury to a gland, while ranulas form in the sublingual gland and may descend into the neck. Furthermore, the influence of age and sex must be considered; for example, Sjögren's syndrome occurs more frequently in women during menopause, accompanied by xerostomia and alterations in salivary composition.

The Contribution of Giuseppe Ortonzo Giannuzzi: Discovery of Serous Demilunes and Early Functional Histology. The Italian histologist and physiologist Giuseppe Ortonzo Giannuzzi (1811–1876) is primarily renowned as the discoverer of specialized morphological structures later named the "demilunes of Giannuzzi" (semilunes of Giannuzzi).

He first described these crescent-shaped formations within the acini of mixed salivary glands (most notably the submandibular and sublingual glands). These structures are located peripherally to the mucinous cells and consist of darkly stained serous cells [8].

It is crucial to note that this discovery was made before modern concepts of cellular functional polarity, acinar polymorphism, and dynamic secretory mechanisms became universally accepted.

Methodologically, Giannuzzi's research was distinguished by the innovative use of microscopy on frozen sections, which allowed for the preservation of the cells' natural state without significant structural degradation. He also performed dye injections and utilized chemical fixatives to achieve better contrast between different cell types.

Functionally, he interpreted "Giannuzzi's crescents" as actively secreting serous cells that produce the aqueous component of saliva with a high enzyme content (primarily α -amylase). [5]. He emphasized their connection with the intra-acinar canaliculi and their role in regulating the flow of secretion. These ideas prefigured later concepts regarding the polarity of cells in endo-exocrine glands.

Although alternative interpretations emerged later for instance, the view that the "demilunes" are fixation artifacts caused by mucin expansion pushing serous cells to the periphery of the acinus [5] Giannuzzi's discovery fundamentally established the morphological basis for distinguishing between serous, mucinous, and mixed types of acini. Today, the term "demilunes of Giannuzzi" is universally accepted in histology and is taught as a basic structural component of mixed salivary glands in medical universities.

Thus, the historical and scientific analysis of Giuseppe Oronzo Giannuzzi's research demonstrates that his contribution to the study of salivary glands is fundamental to the development of functional histology, anatomy, and the physiology of exocrine glands. Despite differing scientific styles, national schools, and methodological approaches, the researcher significantly expanded the understanding of the microscopic structure and functional dynamics of salivary glands, laying the groundwork for further research in morphology, histochemistry, and clinical medicine.

By being the first to describe crescent-shaped serous cells at the periphery of mixed acini, Giuseppe Oronzo Giannuzzi initiated a differentiated approach to the analysis of salivary gland microstructure. His discovery was pivotal in forming the modern classification of acini into serous, mucous, and mixed types, as well as in understanding the specificity of their secretory activity. Despite certain later criticisms regarding the potential artifactual nature of the demilunes, they remain a universally recognized element of histological nomenclature.

Serous Demilunes: A Morphological Phenomenon or a Histological Processing Artifact? One of Giuseppe Oronzo Giannuzzi's key discoveries in the field of salivary gland histology was the description of serous "demilunes" in the submandibular glands. He observed that specialized crescent-shaped serous cells, forming distinct "demilunes," are situated at the terminal portions of mucous acini. This observation was of great significance for the early understanding of the heterogeneity of cell populations within glandular structures and their potential functional specialization.

However, subsequent studies have indicated that the appearance of these "demilunes" is partially or entirely related to the methodology of histological tissue processing [8]. Traditional methods of fixation and dehydration used in the 19th century caused the displacement of mucous and serous cells, thereby forming the visible crescents. Experiments utilizing modern rapid-freezing techniques and osmium fixation have demonstrated that when the natural spatial arrangement of cells is preserved, these formations are not always observed.

Nevertheless, electron microscopic studies (Igbokwe C.O., 2018; Martinez et al., 1982) have shown that some degree of peripheral localization of serous cells in mixed acini does indeed exist, particularly in the submandibular glands of animals, which supports the hypothesis of structural heterogeneity within acini [9].

Thus, the serous "demilunes of Giannuzzi" can be viewed as a morphological phenomenon that partially reflects the natural organization of cells but is significantly manifested as an artifact of classical histological preparation methods. This finding is essential for historical and scientific analysis, as it demonstrates how the technical limitations of an era influenced the formulation of structural concepts and underscores the necessity for a critical approach to classical histological data.

While the serous demilunes of Giannuzzi remain an important historical and scientific phenomenon in salivary gland morphology, contemporary research suggests that their observation was dependent on fixation methods and, therefore, they may not always reflect true histological reality.

Historical and Contemporary Perspectives on Giannuzzi's Serous Demilunes. The study of the morphological characteristics of the major salivary glands over the past two centuries has evolved from classical light microscopy descriptions to modern electron microscopic and molecular research. A historically significant milestone in this field was the discovery by Giuseppe Oronzo Giannuzzi, who in 1865 described serous "demilunes" (*lunulae*, crescents of Giannuzzi) at the periphery of mucous acini in mixed salivary glands [30]. His observations laid the groundwork for the establishment of standard histological terminology and have been cited for

decades as the classical description of glandular tissue morphology. The subsequent development of the histology of major salivary glands was closely linked to the refinement of fixation and microscopy techniques. Textbooks and atlases by Junqueira, Ross & Pawlina, Kierszenbaum, Dellmann, and others consistently reproduce the structures described by Giannuzzi, presenting them as a characteristic component of mixed glands [9].

In these sources, serous cells are presented as pyramidal elements with a basally located nucleus, a well-developed rough endoplasmic reticulum, a Golgi apparatus, and numerous electron-dense secretory granules containing amylase and other enzymes. In mixed acini, they typically form "cap-like" clusters adjacent to mucous cells, forming the so-called serous demilunes.

However, with the advancement of electron microscopy, controversies emerged regarding the interpretation of this phenomenon. A number of studies, notably the work of Yamashina et al. (1999), demonstrated that when utilizing rapid freezing and freeze-substitution methods, serous cells are positioned side-by-side with mucous cells, and the characteristic "crescent-shaped" structures are either absent or exhibit a much less pronounced configuration [5]. This provided grounds to suggest that the phenomenon of "Giannuzzi's demilunes" is, at least in part, an artifact caused by classical chemical fixation methods, which trigger the swelling of mucous granules and the subsequent mechanical displacement of serous cells to the periphery of the acinus.

Regardless of this, most modern morphological sources continue to represent serous demilunes as an educational and diagnostically convenient landmark. The literature emphasizes that the morphology of mixed glands exhibits species-specific differences and depends on the topography of the glands themselves (submandibular, sublingual, or parotid). Functionally, serous cells remain critically important elements that ensure the secretion of enzymes and antimicrobial proteins, independent of their exact architectonics within the acinus.

Summarizing the available data, it can be asserted that Giannuzzi's discovery was of fundamental importance for the development of salivary gland histology and laid the groundwork for ongoing scientific discussion. Modern research has clarified that the shape and localization of serous cells depend not only on the biological characteristics of the tissue but also on the fixation methods applied. Thus, the phenomenon of serous demilunes cannot be regarded exclusively as an artifact or as an unconditionally stable anatomical structure; it is a combination of a historically described morphological fact and a methodological effect.

Consequently, Giannuzzi's historical observations and their modern interpretation indicate the necessity of integrating classical morphology with cutting-edge research methods, such as electron microscopy, immunohistochemistry, and rapid-freezing techniques. This approach allows for the avoidance of one-sided interpretations and fosters a more precise understanding of the morphofunctional organization of the major salivary glands.

Conclusion

The literature analysis confirms that the major salivary glands of humans and rodents share a similar general anatomical and histological organization, yet they exhibit species-specific differences in topography, acinar structure, and ductal systems. Giuseppe Oronzo Giannuzzi's discovery of serous "demilunes" became fundamental for the classification of acini into serous, mucous, and mixed types and provided the basis for understanding secretory dynamics. Although modern methods suggest that some demilunes may be artifactual, their morphological reality is supported by electron microscopic studies. In summary, this historical and contemporary analysis facilitates a deeper understanding of the morphofunctional organization of the salivary glands and holds applied significance for physiology, pathophysiology, and clinical medicine.

Thus, Giannuzzi's legacy is not only a historical asset but also a source of inspiration for modern morphologists, anatomists, physiologists, and clinicians working in the fields of salivary glands, secretion, and cell biology. His discovery remains an integral part of the scientific paradigm that defines our current understanding of the structure and function of exocrine glands.

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Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Hryn V.H. <https://orcid.org/0000-0001-5894-4416>, Tikhonova O.A. <https://orcid.org/0000-0001-7796-0809>, Piliuhin A.V. <https://orcid.org/0000-0003-0601-8036>, Tarasenko Ya.A. <https://orcid.org/0000-0003-2296-9464>, Fedorchenko I.L. <https://orcid.org/0000-0002-7840-887X>, Hryn M.V. <https://orcid.org/0009-0001-0962-4069>, Shevchenko O.M. <https://orcid.org/0000-0002-5829-2048>.

Article received: 10.07.2025