

First evidence of histological and histochemical intraspecific differences in salivary glands in Brown Norway and albino Wistar rats*

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Abstract: The size of salivary glands, acinar structure, and secretion particularities differ from one species to another and are influenced by diet and environment. In order to investigate whether there are histological or histochemical differences in the salivary glands of animals from the same species, bred in identical conditions of environment and diet, we assessed two laboratory rat strains. The salivary glands from two rat strains were processed for histological and histochemical investigations. Histologically, we noticed differences in the parotid gland: seromucous acini were present in Brown Norway rat and typical serous ones in Wistar rat. Histochemically, we observed that cells in the parotid gland present a moderate PAS-positive reaction only in Wistar rat. In the mandibular gland, the size and shape of the acini were similar, whereas the cytoplasm of acinar and granular duct cells was PAS-positive in both rat strains, but with different intensities. In the sublingual gland, both PAS and Alcian blue reactions were positive in the two rat strains, with a higher intensity in the Brown Norway strain. Our study highlighted intraspecific histological and histochemical differences in salivary glands from two rat strains bred and fed in identical conditions.

Key words: Brown Norway rat, mandibular, parotid, sublingual, Wistar rat

1. Introduction

Salivary glands are distinct symmetrical structures, annexes of the digestive tract, situated at a certain distance from the oral cavity. In mammals, major salivary glands are represented by three pairs of organs, which functionally cooperate to produce the saliva necessary in the oral cavity (1–3). Each gland produces an individual secretion in order to complete other glands' secretion (4,5).

Upon comparison of the salivary glands in hundreds of species, no single description can be applied to all mammals (6). The lack of conservation of the salivary glands' structure is significant because it shows that their evolution is not random but correlates with the way of feeding or the environment in which the animals live (7).

Utilization of laboratory animals, particularly rodents, for technical and bioethical reasons in studies on salivary glands for human and other benefits requires a good understanding of both the histological and the biochemical structure. Most researchers are not aware of the structural

differences among humans and rodents because most of the information available only describes salivary glands in humans (8). Thus, a wide database is needed for researchers working in this field.

The size of these organs, the acinar structure, and characteristics of the secreted saliva (serous, mucous, or mixed) differ from one species to another and is influenced by diet (9–12). In the case of the same gland, the salivary secretion is different depending on the way of living and feeding. For instance, the submandibular gland is mixed in rats (13), rabbits (14), miniature pigs (15), and hamsters (16), but serous in ferrets (17) and koalas (18). Ikpegbu et al. (19) affirmed that the mandibular gland in African giant pouched rat (*Cricetomys gambianus*) comprises two distinct regions, separated by a fine connective tissue. One region contains mostly serous cells, while the other has predominantly mucous cells.

The secretion product of some of these glands can be utilized by certain animals for different purposes. In

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snakes, the salivary glands are modified to produce venom for self-defense, while in ants, the saliva can represent nourishment for the eggs (10,20). Most researchers report that differences can exist in the histology and histochemistry of salivary glands of rodent species and that these would be due to the environment in which the animals live (21,22). Some histochemical studies regarding the mucopolysaccharides or enzymes in salivary glands in animals with different diets showed that the secretion of salivary glands is linked to alimentation habits (21).

Some histological and histochemical differences were also observed, but less obvious, in species that live in the same environmental conditions and have similar diets. Moghaddam et al. studied two rodent species, *Allactaga elater* and *Jaculus blanfordi*, which live in arid or semiarid areas, with the same environment and food conditions. They noticed that the mandibular gland in *Allactaga elater* presents serous and mucous acini, while *Jaculus blanfordii* has only serous acini (21). These authors claimed that in this case, differences can appear due to other factors, besides environment and diet.

We did not find information in the scientific literature we consulted regarding the existence of histological and histochemical differences in salivary glands among animals from the same species, raised in the same environmental and dietary conditions. In order to assess if such differences exist or not, we conducted histological and histochemical investigations of the salivary glands of rats from two different strains.

2. Materials and methods

The study was approved by the Scientific Research Ethics Committee of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca and was conducted in accordance with the European legislation. The biological material was represented by two different rat strains: albino Wistar rats and Brown Norway rats. We used five animals from each strain, all males, coming from the biobase of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca. Both rat strains were kept in the same environment and fed with the same nourishment. The environmental conditions were constantly controlled: 22–23 °C, around 60% humidity, and a 12-h dark/light cycle. The rats received ad libitum standardized rodent granulated diet (Cantacuzino Institute, Romania) and fresh water. Animals were sacrificed by prolonged exposure to an inhaled anesthetic (Aerrane isoflurane, Baxter S.A.). After euthanasia, we harvested the major salivary glands in order to conduct histological and histochemical investigations. The samples were fixed in 10% buffered formalin for 5 days. We subsequently dehydrated the tissues in increasing concentrations of ethanol (70°, 95°, and absolute), clarified them in n-butanol, and embedded

them in paraffin. We sectioned the samples at a thickness of 5 µm, using a microtome (Leica rotary microtome). For histological investigations, the sections were stained with hematoxylin and eosin (H&E). For the histochemical ones, we performed periodic acid–Schiff (PAS) staining and Alcian blue (pH 2.5) reactions (23). The histological and histochemical slides were examined under an Olympus BX41 light microscope equipped with a digital camera (Olympus E-330) for image capturing, and for the subsequent image processing, we used Adobe Photoshop CS2 software.

3. Results

In Brown Norway rat, the parotid gland contains only one type of acini, which are polymorphic, both in shape and size, without any obvious structural differences between acinar cells (Figure 1). The nucleus of acinar cells is spherical, while the cytoplasm presents a more or less vacuolar aspect. In albino Wistar rat, the parotid gland contains acini with comparable polymorphism to that in Brown Norway rat concerning their shape and size. All acini are also of the same type, but different from the ones in Brown Norway rat in the sense that here the cytoplasm of acinar cells appears more uniform, mainly granular (Figure 2). There are also differences concerning the tinctorial affinity of the cytoplasm, which is clearly more acidophilic here.

In Brown Norway rat, the parotid gland contains glandular acini, whose cells do not present PAS-positive material in their cytoplasm (Figure 3). In albino Wistar rat, the parotid gland presents some differences in comparison to Brown Norway rat, in the sense that the cytoplasm of glandular cells presents PAS-positive material, with different intensities from one acinus to another and even from one area to another (Figure 4). This highlights the fact that cellular secretion in albino Wistar rat is not identical to that in Brown Norway rat.

In Brown Norway rat, the mandibular gland contains very discreet PAS-positive material in the cytoplasm of glandular cells in all acini, with little differences among them. The cytoplasm of cells lining the granular ducts appears PAS-positive also, but it seems to have a higher intensity in comparison to that in cells from glandular acini. In albino Wistar rat, the mandibular gland is a bit different from that in Brown Norway rat, even if there is also PAS-positive material present here in both acinar and granular ducts cells. The difference is given by the intensity of the reaction, which in Wistar rat is higher in the cytoplasm of acinar cells and lower in cells lining the granular ducts.

In Brown Norway rat, the sublingual gland appears intensely PAS-positive, with a uniform reaction throughout the whole section surface (Figure 5). The situation is

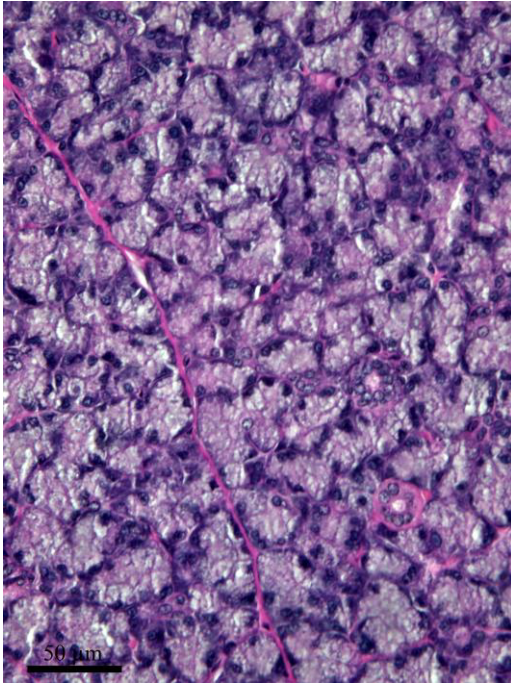


Figure 1. Parotid gland in Brown Norway rat (H&E stain).

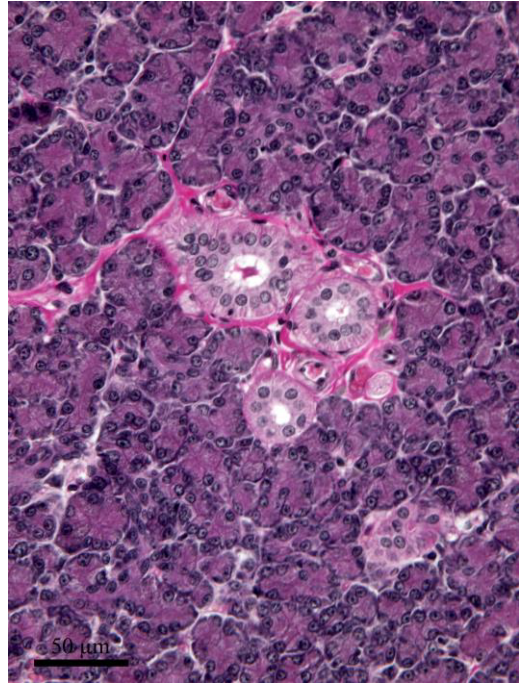


Figure 2. Parotid gland in albino Wistar rat (H&E stain).

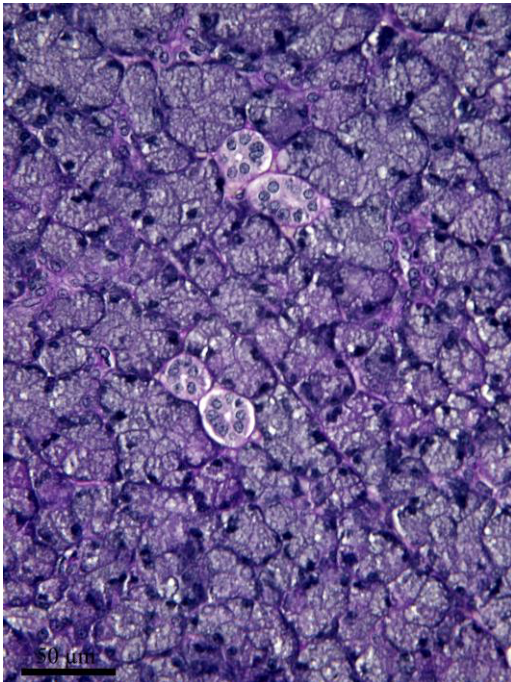


Figure 3. Parotid gland in Brown Norway rat (PAS).

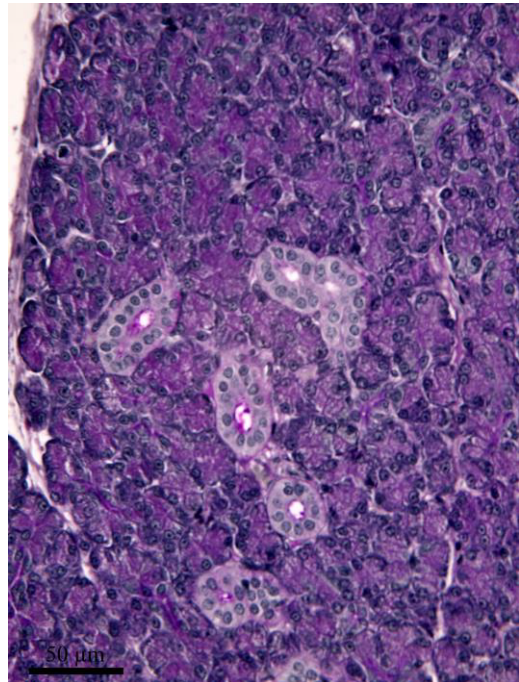


Figure 4. Parotid gland in albino Wistar rat (PAS).

somewhat comparable in the sublingual gland of Wistar rat, which appears PAS-positive, but the reaction intensity is more diminished here (Figure 6).

In Brown Norway rat, the sublingual gland presents moderate positive Alcian blue reaction in intensity, but

relatively uniform (Figure 7). In Wistar rat, the sublingual gland also presents positive Alcian blue reaction with a significantly fainter intensity in comparison to Brown Norway rat (Figure 8).

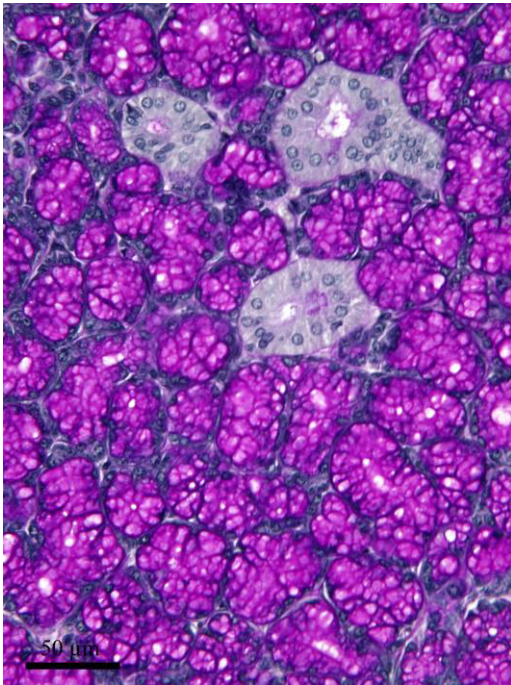


Figure 5. Sublingual gland in Brown Norway rat (PAS).

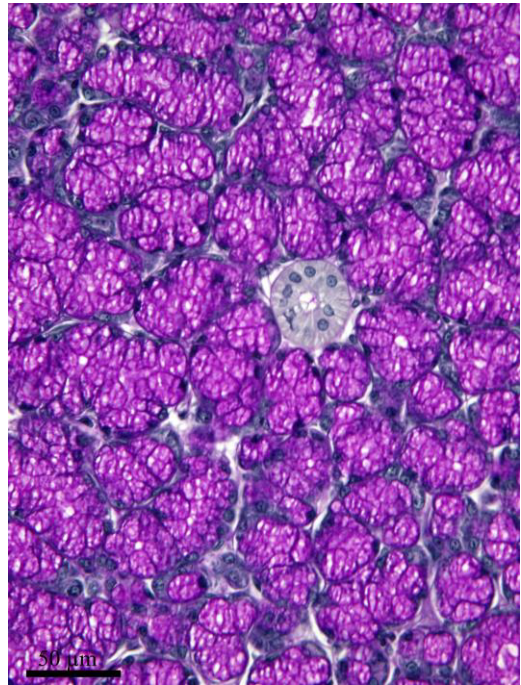


Figure 6. Sublingual gland in albino Wistar rat (PAS).

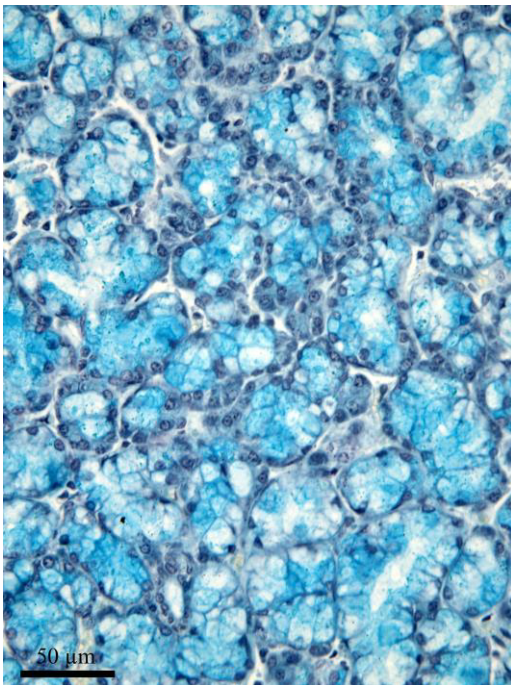


Figure 7. Sublingual gland in Brown Norway rat (Alcian blue).

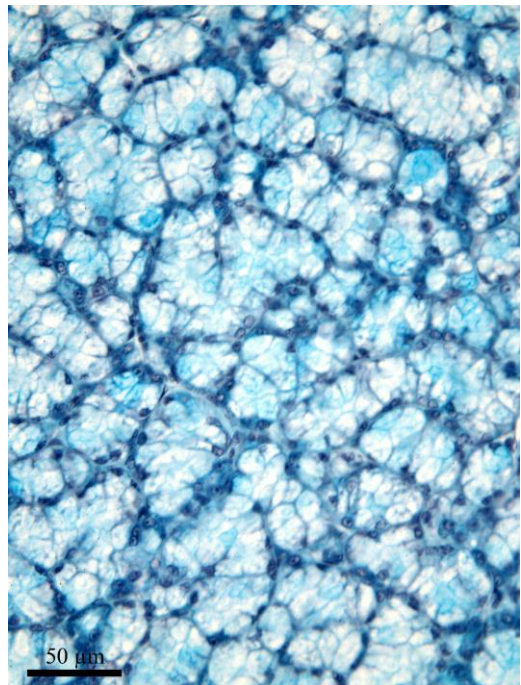


Figure 8. Sublingual gland in albino Wistar rat (Alcian blue).

4. Discussion

In the case of major salivary glands in the rats studied here, we highlighted both comparable and more or less different aspects between the two rat strains. The common aspects

in the case of the parotid gland are given by the fact that it contains only one type of acini, polymorphic in shape and size, and the nucleus of acinar cells is spherical. In Brown Norway rat, the cytoplasm appears more or less vacuolar,

while in Wistar rat it appears uniform, mainly granular and clearly more acidophilic. The vacuolar aspect of the cytoplasm led certain authors to catalogue such acini as seromucous (2). If we assess this aspect, we can state that in Brown Norway rat, from a morphological point of view, acini are seromucous, while those in albino Wistar rats are serous. Our results are not comparable to those of other authors, who stated that parotid glands in rat, hamster, Guinea pig, rabbit, rhesus macaque, and pig are highly similar regarding their morphology and histochemistry (14).

We also observed differences between acinar cells in the two rat strains in the histochemical reactions we performed, and especially in PAS reactions. Thus, in the cytoplasm of acinar cells, we highlighted PAS-positive substances of moderate intensity only in Wistar rat, while in the case of Brown Norway rat, the reaction was negative. Based on these aspects, we can state that the secretion of cells in albino Wistar rat parotid gland is different, to a certain extent, from that in Brown Norway rat. Our results also differ, from this point of view as well, from those obtained by other authors, which highlighted a detectable quantity of PAS-positive material in the striated ducts from rat parotid gland (2). The authors did not mention if the PAS-positive material was observed in the lumen of the striated ducts or in the cytoplasm of the cells lining the duct. If they observed it in the lumen of the striated ducts, it is possible that it originated from the acini. Cells lining the striated ducts from the parotid of both rat strains studied by us were PAS-negative. On the other hand, we highlighted PAS-positive material in the acini of the parotid gland in albino Wistar rat. If the authors mentioned above worked on Wistar rats, then the PAS-positive secretion found by them in the striated ducts was originating from the acini.

In the case of the mandibular gland, there are common aspects, but also particular ones for each rat strain studied. The common ones are again linked to the size and shape of the acini, but also of the excretory ducts, which are comparable in the two rat strains. In PAS reaction, some differences appear. In Brown Norway rat, the intensity of PAS reaction is somewhat more pronounced in the cells from granular ducts than in acinar cells, while in albino Wistar rat this situation is reversed. This suggests that the secretion of the mandibular gland is different to a certain extent from one rat strain to another. Other authors reported that the mandibular gland in rat, mouse, and hamster is similar in structure and histochemical properties (13). Our investigation highlighted that intraspecific differences appear between the studied rat

strains; thus, our results differ from those of the above-mentioned authors.

In the case of the sublingual gland, the utilized histochemical reactions showed that there are similarities between the two studied rat strains, but also dissimilarities. The similarities were given by the fact that in the case of both PAS and Alcian blue reactions, the sublingual gland of both rat strains was positive. The dissimilarities consisted in the intensity of the reaction, which was more pronounced in the case of Brown Norway rat for both PAS and Alcian blue reactions. In other words, the sublingual gland in Brown Norway rat is more active than that in albino Wistar rat.

The results obtained by us highlight the fact that in the case of rats, there are intraspecific histological and histochemical differences in the three major salivary glands. Such differences are known and natural between different species, considering the particularities of food and environment in which every species lives, but the presence of differences in animals from the same species belonging to different strains is somewhat surprising, especially if we bear in mind the fact that the rats from both strains are laboratory animals bred and kept in the same environment (22–23 °C, 60% humidity, 12-h dark/light cycle) and fed with the same nourishment (standardized rodent granulated diet). In our opinion, the presence of such differences in animals from the same species could only be due to the different conditions in which each strain in part developed and not necessarily the actual diet. In other words, we consider these differences as adaptive structures for the particular habitat and the nature of alimentation from the time when each rat strain was formed. Even though we did not find any reports on the existence of histological and histochemical differences in salivary glands between human populations who live in different environments and have different diets, we consider that is highly possible that they exist and it is only a matter of time until they are identified.

The histological and histochemical aspects highlighted in the salivary glands from two different rat strains demonstrate that not only interspecific differences but also intraspecific ones can arise, even in animals held in the same environmental and food conditions.

As there are no differences regarding the breeding and feeding conditions, we consider that the existing differences among the salivary glands in animals from the same species represent adaptive structures for the particular habitat and the nature of alimentation from the period when each rat strain was formed.

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