PhD THESIS

Morphological and histochemical study of the digestive system in the Wistar rat

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

The digestive system is very complex, being made up of a large number of organs, each specialized to participate in one of the stages of digestion, depending on the anatomical arrangement within the system.

The relatively large differences between animals, related to size, type of diet, temperament, degree of adaptation to different pedoclimatic conditions, etc., mean that the organs that make up the digestive system are not identical in all species, but only as anatomical segments.

THESIS STRUCTURE

The PhD thesis entitled "Morphological and histochemical study of the digestive system in the Wistar rat" comprises 165 pages and contains 102 figures (19 macroscopic and 83 microscopic). This was written according to the methodologies for writing doctoral theses and is structured in two parts:

The **first part** of the thesis is presented in 34 pages and organized in two chapters.

Chapter I, entitled "*The rat - general aspects*" contains information on the origin of rats, species of rats, laboratory rats, behavior, analyzers, raising, as well as maintenance and nutrition of the laboratory rat.

Chapter II, entitled "*Anatomical and physiological features of the laboratory rat*" contains information about the anatomical segments of the rat and the conditions necessary for its growth.

The **second part** of the thesis is presented in 101 pages and divided into 7 chapters.

It is structured in 7 chapters and includes: objectives, materials and methods used for macroscopic, microscopic and histochemical morphological research of all the components of the digestive system in the laboratory rat. The actual research ends with the general conclusions, followed by the aspects of originality and innovative contributions of the thesis.

OBJECTIVES OF THE PAPER

- the macroscopic study of the organs of the digestive system in the Wistar rat, in order to capture any particular aspects;

- the microscopic study of the organs of the digestive system in the Wistar rat, to check if there are adaptive histological structures, due to the change in diet;

- the histochemical study of the existing glandular structures in the digestive system, in order to estimate the type of secretion of each individual cell type;

MATERIALS AND METHODS

The *animals used* in this study are represented by 10 Wistar adult rats, raised in the biobase of the Faculty of Veterinary Medicine in Cluj-Napoca.

For anatomical investigations, the usual dissection was performed and the following organs were examined, described and photographed: oral cavity, teeth,

tongue, esophagus, stomach, small intestine, large intestine, major salivary glands, liver and pancreas.

For histological and **histochemical investigations**, fragments were collected from the following organs: tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, salivary glands, liver and pancreas. For general histological investigations, staining with the Goldner trichrome method was practiced, and for histochemical ones, the PAS reaction, for neutral mucosubstances and alcian blue staining, for acidic ones.

In **chapter 5** entitled "*Anatomical features of the digestive system in the Wistar rat*" the inventory of anatomical aspects related to the shape, size and topography of the organs of the digestive system was performed. In order to achieve this goal, I proposed the following objectives:

- performing anatomical dissection in mature rats raised in biobase conditions, to assess all the components of the digestive system and the relationships with the neighboring structures;

- capturing of possible particular aspects at the level of the digestive organs, which could be important in the research activity on the digestive system.

The tongue of the Wistar rat is attached from the level of the epiglottis to the third molar, where it becomes free (unattached) and extends to the lower incisors. Three types of filiform papillae are present on the dorsal surface, short and strongly curved anteroposteriorly, in the anterior half, highly developed and slightly curved postero-anteriorly on the protuberance, and medium, slightly curved postero-anteriorly and with branched tips, on the lingual body.

Rats are monophyodont animals, which means they develop only one set of teeth throughout their lives. The dental formula in the rat is 2x (I1/1, C0/0, PM0/0, M3/3) =16. In this species, there are no canines and premolars, and between the incisors and molars there is a region without teeth, which is called a diastema.

The esophagus in the rat is a tubular formation that connects the pharynx to the stomach. It consists of three segments: cervical, thoracic and abdominal. The cervical segment is located dorsal to the larynx, the thoracic follows the chest, to the left of the trachea, and passes through the esophageal hiatus in the diaphragm, slightly to the left of the midline. The abdominal esophagus is short and enters the stomach on the small curvature near the limiting ridge (pleated border).

The stomach is located on the left side of the proximal abdomen, partially covered by the left lateral lobe of the liver. The gastric mucosa is divided into two regions: proventricular and glandular. The proventriculus makes up about two-thirds of the stomach and lies to the left of the limiting ridge, encompassing the foramen cardia. This region is lined by mucosa whose epithelium is keratinized stratified squamous. The glandular stomach of the rat is divided into three anatomical segments: cardiac, fundic, and pyloric.

The small intestine is the longest portion of the gastrointestinal tract, reaching 170 cm in the rat. It is arranged from the pyloric sphincter to the ileo-cecal valve, being made up of three distinct regions: duodenum, jejunum and ileum. The duodenum is the shortest, and the rest of the small intestine is divided between the jejunum, which is the proximal two-fifths, and the ileum, which is the longest segment and comprises the distal three-fifths.

The large intestine is located from the ileo-cecal valve to the anus and consists of the cecum, colon, and rectum, provided externally, with the anus.

The cecum has the shape of a relatively large, blind, curved sac, representing up to a third of the length of the large intestine.

The colon in the rat lies from the cecum, with the first segment, called the ascending colon, and continues with a relatively short segment - the transverse colon, and this continues with the descending colon, which ends at the level of the rectum, which opens to the outside, through the anal opening.

The liver varies in weight depending on the body size of the animals, does not have a gall bladder and consists of four lobes: left, median, right and caudate.

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The rat pancreas is a diffuse organ, dispersed throughout the mesentery adjacent to the duodenum. This pancreas model does not allow the recognition of anatomical regions from other species, called head, neck, body and tail.

Chapter 6 entitled "*Histological assessment of the digestive system in the Wistar rat*" covered histological investigations of the main components of the digestive system in the laboratory rat. To achieve this goal, I proposed the following objectives:

- checking the structure of all the components of the digestive system in the Wistar rat by histological study;

- identifying the possible particular structural aspects of the components of the digestive system in the Wistar rat.

The tongue is covered by the lingual mucosa consisting of surface epithelium and chorion. The surface epithelium is keratinized stratified squamous and has characteristic projections called lingual papillae, which are present on the entire dorsal surface, partially on the margins and absent on the ventral surface. The stratified squamous epithelium covers the entire surface of the tongue, namely the dorsal side, where the epithelium covering the papillae is intensely keratinized, but also the one between the papillae is also keratinized, moreover the epithelium on the ventral side shows a high degree of keratinization.

The esophagus has a somewhat comparable caliber in this species throughout its length, except for the last portion, where it appears with a slightly larger diameter. The wall of the esophagus consists of the four tunics characteristic of the digestive tube: mucosa, submucosa, muscular and serous. The epithelium is stratified squamous with some degree of keratinization, the chorion consists of relatively dense connective tissue, and the muscularis mucosae is discrete and forms a discontinuous layer of smooth muscle fibers.

The stomach of the Wistar rat contains a proventricular and a glandular segment, which is divided into cardiac, fundic, and pyloric regions. The two segments - proventricular and glandular, are separated by the limiting ridge. The proventricular portion and the limiting ridge are covered with squamous stratified epithelium, which continues that of the esophagus. The cardiac region contains a relatively thick mucous membrane in which numerous glands are present, which, in appearance, do not appear to consist entirely of one type of glandular cells, the proportion of them changing more and more as we approach the fundal region. The fundus region presents typical mucosa, which occupies more than half of the total thickness of the gastric wall and presents numerous glands arranged closely together and with typical structure, namely parietal cells and principal cells. The pyloric region is well represented, it contains numerous glands, but a smaller number than in the fundic area. Particular is the fact that the glands in this region are simple tubular and much straighter than in most mammalian species.

The duodenum has relatively high villi and well represented in density, and the epithelium is simply prismatic, lining the villi, the space between them, and the epithelial component of the glands. The organ contains the two types of glands specific to the duodenum, namely Lieberkuhn glands, in the depth of the mucosa, and Bruner duodenal glands, in the submucosa. Although Bruner's glands are relatively well represented, they occupy about half the area occupied by Lieberkuhn glands.

The jejunum presents numerous villi, higher than in the duodenum and with a digitiform appearance. This segment presents only one type of gland - Lieberkuhn glands, of medium length. In appearance they are simple and straight tubular glands, consisting of prismatic cells and goblet cells.

The ileum contains shorter, thicker and slightly sparser villi than in the jejunum. The glands are rarer than in the jejunum, but they are comparable in size, appearance and structure.

The cecum has the four tunics characteristic of the digestive tube, but has a significantly thinner wall than the segments of the small intestine. The thickness of the mucosa is comparable at the base and body of the cecum, but slightly thinner at its tip. The mucosa contains glands, but significantly fewer than in the small intestine, the fewest recorded at the level of the cecal apex. The glands contain prismatic cells, goblet cells - more like in the small intestine, and in the deep third there are pyramidal cells, which appear to be DCS cells.

The colon has a relatively similar structure in its three segments - ascending, transverse and descending. The mucosa contains longer glands than in the small intestine, but in terms of density, they are less here. The glands contain enterocytes, numerous goblet cells, and a small number of DCS cells. The musculature of the mucosa is significantly thicker than in the small intestine. The musculature is arranged in two layers and does not present muscular taenia.

The major salivary glands in the Wistar rat are those found in all mammalian species, namely the parotid, mandibular and sublingual.

The parotid consists of serous acini, of small size, in which the glandular cells present spherical nuclei and delimit with their apical pole, a very small lumen, proof of the fact that the secretion of these cells is fluid, serous.

The mandibular and sublingual glands appear in this species arranged closely together, practically separated by a layer of thin connective tissue, but which clearly separates the two glands, so that there are no interpenetrations of glandular tissue. In the mandible the acini are all of the same kind, namely serous acini, but they do not seem to be identical to those in the parotid. The excretory ducts present, in addition to the segments known in other mammalian species, particular ducts, called granular ducts, arranged between the intercalary ducts and the striated ducts. The sublingual has a structure comparable to that existing in most mammals.

The liver of the Wistar rat has a classic structure, consisting of hepatic lobules, separated by a very small amount of connective tissue.

The pancreas has a structure comparable to that existing in other mammalian species - it contains an exocrine component formed by acini and excretory ducts, and an endocrine component, represented by polymorphic islets of Langerhans.

Chapter 7 entitled "*Histochemical assessment of mucin-secreting cells in the organs of the digestive system in the Wistar rat*" aimed at inventory the existing mucin-secreting cells in the digestive system of the Wistar rat, their arrangement in the

structure of each organ, as well as the type and intensity of secretion for each cell type. In order to achieve this goal, we have established the following objectives:

- establishing an inventory of mucin-secreting cells existing in the organs of the digestive system in the Wistar rat;

- highlighting the type of mucins and the intensity of secretion of all glandular cells existing in the organs of the digestive system.

Through the PAS reaction, I followed the glandular cells in all organs, to identify those that secrete neutral mucins.

The acini of the von Ebner gland are positive on the PAS reaction at an intensity rated by us as ++. However, the PAS-positive material in the cytoplasm of acinar cells appears different from that identified in mucous acini cells, in Weber's gland, or in the sublingual, both in terms of the intensity of the reaction and the shade at which it stains. This leads us to believe that the PAS-positive material in the cytoplasm of von Ebner's gland acinar cells is not represented by neutral mucins, but by other PAS-positive substances.

The acini of Weber's gland are intensely PAS positive (+++), and PAS material can also be identified in the lumen of the excretory ducts. They have a wide lumen, which suggests that it is relatively viscous mucus, the appearance of which is somewhat runny. The degree of viscosity and the runny appearance seem to be an adaptation to the needs of the area where this mucus acts (root of the tongue, pharynx and esophagus).

The acini of the parotid gland are also PAS positive, but of low intensity, about half that of the acini of the von Ebner (+) gland. This fact suggests that the acinar cells of the parotid gland synthesize PAS-positive substances, but in a relatively low amount, and they do not appear to be mucins, but other PAS-positive substances.

The acini of the sublingual gland are PAS positive (++), and the color of the acinar cells suggests that they are neutral mucosubstances. The slightly lower intensity of the color compared to the acinar cells of the Weber gland, suggests that the intensity of the secretion is slightly higher in the Weber gland compared to the sublingual gland.

In the mandibular gland, the appearance is very particular, in the sense that the acini here are all PAS negative, but in the cytoplasm of the cells of the glandular ducts, there is discrete material, PAS positive, in a quantity that varies relatively much, from one cell to another. From the color shade, it appears that these PAS positive substances are not neutral mucins.

In the esophagus, the PAS reaction detected PAS-positive substances, only in the lumen of the organ, which proves that they are here in transit, and come from the level of the oral cavity.

Regarding the stomach, the situation is similar in its three anatomical subregions, only regarding the cells lining the gastric mucosa and the crypts, which all appear intensely PAS positive. The components of the mucosal depth differ depending on the subregion we are referring to. The cells of the cardiac glands and those of the fundic glands are PAS negative, on the other hand, the pyloric glands all present a positive PAS reaction, the lumen of the glands being entirely occupied by PAS positive material.

In the duodenum, the goblet cells in the villous epithelium and those in the walls of Lieberkuhn glands are PAS positive. Bruner's gland cells are also PAS positive, but the intensity of the reaction is obviously lower. This aspect suggests that both cell categories synthesize neutral mucosubstances.

In the jejunum, the only PAS-positive cells are the goblet cells, and the intensity of the reaction is similar in the cells of the villous epithelium and those of the walls of

the Lieberkuhn glands. Also, goblet cells in the jejunum are comparable in PAS reaction intensity to those in the duodenum.

In the ileum, the situation is similar to that in the jejunum, in that goblet cells are the only PAS-positive structures, and the intensity of the reaction is comparable, regardless of their location.

In the cecum, there are two types of PAS-positive cells, namely goblet cells and DCS cells, present in varying numbers at the base of Lieberkuhn glands. The number of DCS cells is not very high in any of the segments of the check, and the intensity of the reaction is relatively weak.

In the three segments of the colon there are PAS positive cells, arranged both in the surface epithelium and in the walls of the Lieberkuhn glands. In the surface epithelium and in the upper half of the glands, there is only one type of PAS-positive cells, namely goblet cells, and the intensity of the reaction is average. In the deep half of the glands, in addition to goblet cells, there are DCS cells. These are also PAS positive, but the intensity of the reaction is significantly lower than the goblet ones. It is found that both types of cells secrete neutral mucins, but goblet cells secrete obviously higher amounts than DCS cells.

I also followed the glandular cells on the alcian blue staining, from the level of the oral cavity, to the colon. Among the minor salivary glands, the cells of the acini of the Weber gland showed a positive alcian reaction, of medium intensity, and the cells of the von Ebner gland were alcian negative.

Acinar cells of the parotid gland were negative on alcian blue staining.

The acinar cells of the sublingual gland were intensely alcian positive, and the small differences that appear between the cells regarding the degree of mucus loading, seem to be due to the secretory stage in which each cell is.

As for the mandibular gland, no positive alcian reaction was found either in the acinar cells or in those in the walls of the granular ducts.

Cells lining the gastric mucosa and crypts were alcian negative in all three regions of the stomach - cardial, fundic and pyloric. The cells in the walls of the glands in the cardia region and in the fundus region also showed a negative alcian reaction. The only region where some of the cells showed a weak alcian positive reaction, was the pyloric region. It should be noted that it is a limited number of cells, located in the deep third of the pyloric glands.

In the duodenum, only one type of cells positive for the alcian blue reaction, namely the goblet cells, were highlighted, the intensity of the reaction being comparable in the cells from the epithelium of the villi and that of the gland walls. Bruner's gland cells were all alcian negative.

The situation is also comparable in the jejunum and ileum regarding the goblet cells, which are alcian positive of comparable intensity, both in the villous and glandular epithelium.

At the level of the cecum, there are goblet cells and a certain number of DCS cells, both types being alcian positive, with the specification that in the case of DCS cells, the alcian positive reaction is more intense than in the case of goblet cells.

The colon contains positive alcian cells both in the surface epithelium and in the walls of the Lieberkuhn glands, the situation being comparable in the three segments of the colon - ascending, transverse and descending. The most numerous alcian-positive cells are the goblet cells, present both in the surface epithelium and in the walls of the glands, especially in their upper half. In addition to the goblet cells there are alcian

positive cells characteristically clustered in the deep half of the glands. These are DCS cells, and compared to goblet cells, they present a more intense alcian positive reaction.

In **chapter 8** entitled "*General conclusions*" the conclusions that emerged based on the results obtained, following the anatomical, histological and histochemical investigations, were formulated synthetically.

The tongue presents on the dorsal surface short, filiform papillae curved antero-posteriorly to the protuberance, highly developed and curved postero-anteriorly, on the protuberance, medium and curved antero-posteriorly, on the body of the tongue.

Among the major salivary glands, the most developed is the mandibular gland, and the smallest, the sublingual gland, the two being arranged in intimate contact. The parotid gland is arranged in a dorso-lateral position, in relation to the mandibular.

The stomach is elongated and recurved, located on the left side of the abdomen and has a proventricular segment, separated by the limiting ridge from the glandular segment and has three regions: cardiac, fundic and pyloric.

The mucosa of the small intestine presents intestinal villi that differ in shape and size, in the three segments, being tall and leaf-like, in the duodenum, tall and cylindrical, in the jejunum, short and cylindrical, in the ileum.

The liver is well developed in the rat, representing a relatively significant percentage of the body mass, occupies the postdiaphragmatic space, does not present a gall bladder and appears divided into four lobes: left, median, right and caudate.

The musculature of the tongue consists of striated muscle fibers oriented predominantly longitudinally, in the dorsal and ventral superficial layers, and three-dimensional arrangement, with differences from one segment to another, in the middle layer.

The stomach has a very large number of glands, the density of which is highest in the fundus region, then the cardiac and pyloric ones - the fundus ones contain two types of cells, the cardiac ones, several, and those in the pyloric region, only one type.

The intestine presents numerous glands that are of two kinds, in the duodenum - Lieberkuhn and Bruner, and of one kind - Lieberkuhn glands, in the other segments of the small intestine, but also in the large intestine.

The parotid gland has only one type of acini - typical serous, small, with a narrow lumen, the sublingual contains all types of acini, of which the mucous ones clearly predominate, and the mandibular contains serous acini and particular excretory ducts.

The acinar cells in the Weber gland synthesize both neutral mucus, evidenced by the PAS reaction, and acidic mucus, evidenced by alcian blue staining, and from a quantitative point of view, the neutral mucus quantitatively exceeds the acidic one.

Glandular cells in the gastric mucosa produce large amounts of mucins, of which the neutral ones predominate, which are synthesized in all regions of the stomach, and the acidic ones are synthesized only in the pyloric region, being poorly represented quantitatively.

Glandular cells in the duodenum synthesize both categories of mucins. The neutral ones are synthesized by both the goblet cells and those of the Bruner's glands, and they outnumber the acidic ones, which are synthesized only by the goblet cells.

In the jejunum and ileum, only the goblet cells are positive for the two histochemical reactions, and the intensity of the reaction is comparable, so approximately equal amounts of neutral and acid mucins are synthesized here.

Glandular cells are few at the level of the cecum, so mucin secretion is quantitatively reduced, being represented by both neutral and acidic mucins, and quantitatively, the acidic secretion slightly surpasses the neutral one.

The colon contains two types of mucin-secreting cells, of which goblet cells synthesize equal amounts of both categories of mucins, while DCS cells, more acidic mucins, so that acidic mucins slightly outnumber neutral ones.

In **chapter 9** entitled "*Originality and innovative contributions of the thesis*" the most important results obtained from the investigations performed are pointed out.

The anatomical studies provide complete information about the layout, shape and dimensions of each organ of the digestive system in the Wistar rat, and among them we mention:

- The tongue presents, at the level of the protuberance, huge filiform papillae, with the tip tilted postero-anteriorly, i.e. in the opposite direction, compared to the rest of the papillae;
- The papillae on the body of the tongue have a branched tip;
- The stomach has a proventricular and a glandular region, separated by the limiting ridge;
- Intestinal villi are tall and leaf-like, in the duodenum, tall and cylindrical, in the jejunum, short and cylindrical, in the ileum.
- The pancreas is a diffuse organ where the regions found in other mammals cannot be distinguished, namely the head, neck and tail;

The histological studies provide detailed information about the microscopic structure of the organs of the digestive system in the Wistar rat, among which we point out:

- The tongue of the Wistar rat does not contain dispersed glandular acini, but only grouped at the von Ebner and Weber glands;
- There are no glands in the submucosa of the esophagus;
- The proventricular region and the limiting ridge are covered with squamous stratified epithelium comparable to that of the esophagus;
- The fundic glands contain two types of cells, the cardiac ones more, and those in the pyloric region, only one type.
- The mandibular gland has a very particular structure, consisting of serous acini and excretory ducts, which also include granular ducts.
- The glands in the colon contain, in addition to goblet cells, DCS cells;
- Lieberkuhn glands in the colon are longer than those in the small intestine.

The histochemical studies highlighted particular aspects, among which we mention:

- The acinar cells in the Weber gland synthesize both types of mucins, of which the neutral ones predominate;
- Neutral mucins are synthesized in all regions of the stomach, being clearly the majority, and acidic ones, only in the pyloric region, so they are in the minority;
- The glandular cells at the level of the duodenum synthesize both categories of mucins, and the neutral ones quantitatively exceed the acidic ones;
- Approximately equal amounts of neutral and acidic mucins are synthesized in the jejunum and ileum;
- The glandular cells are few in the cecum, they synthesize both types of mucins, and the acid secretion slightly exceeds the neutral one.
- Both types of mucins are synthesized in the colon, and the acidic mucins slightly outnumber the neutral ones.