PhD THESIS

Morphological and histochemical study of digestive system in Chinchilla

SUMMARY OF PhD THESIS

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INTRODUCTION

Chinchilla originates from the arid area of the Cordillean Andes where conditions are very harsh and private, with very high temperature differences from day to night, few rainfalls and less succulent plants adapted to these hostile conditions. Moved to thousands of kilometers on other continents with different climates and conditions, chinchilla has had to adapt to new conditions, different from those in the area of origin and different from one continent to another and from one country to another. In addition to the new pedoclimatic conditions to which it had to adapt, chinchilla had to get used to different plant nutrition in many respects than the one specific to the arid areas of the Cordillean Andes.

STRUCTURE OF THE THESIS

The Ph.D. thesis titled "The morphological and histochemical study of the digestive system at Chinchilla" is on 148 pages and contains 70 figures. It is structured in two parts and has been developed in accordance with the new methodologies for drafting doctoral theses.

The first part of the thesis contains a number of 45 pages and is structured in two chapters.

Chapter I, entitled "Chinchilla - General Aspects", covers aspects of origin, taxonomy, natural habitat, captivity, maintenance, nutrition, reproduction, exterior appearance, personality, and other particular aspects. Also in this chapter is mentioned the economic and social importance of chinchilla.

Chapter II, entitled "Embryology of the Digestive System", includes information about the history of embryology and in particular aspects related to the embryological development of the digestive system, from the primary elements to the final stage of development.

The second part of the thesis consists of 95 pages, it is structured on 8 chapters and contains notions about the working hypothesis, objectives, materials and methods and the research of macroscopic, microscopic and histochemical morphology on the components of the digestive system of chinchilla. This part of the paper ends with the general conclusions and the originality aspects and the innovative contributions of the thesis.

THE PHD THESIS OBJECTIVES

-inventory of aspects of macroscopic morphology of each organ of the digestive system, described or not in the specialty literature;

-the microscopic study of the organs of the digestive system to capture the possible structural adaptive changes in the organs or components of organs;

-histochemical study on organs containing glandular structures to check the types of glandular structures present in each segment of the digestive system and the nature of secretion of each type of glandular cells;

-the indirect microscopic study of the general activity of hepatocytes by quantitating the degree of their loading with the mitochondria.

MATERIALS AND METHODS

The biological material used in this study consisted of ten fresh chinchilla dead bodies and the reagents used were: formaldehyde, potassium dichromate, chromium alum, butyl alcohol, xylene, ethyl alcohol, metal iodide, sodium thiosulfate, concentrated sulfuric acid, glacial acetic acid, periodic acid, normal hydrochloric acid, sodium bisulfite, medicinal charcoal. The colorants used were: hematoxylin, acid fuxin, diamond fuxin, orange G, light green, eosin, alcian blue. Other materials used were: histological paraffin and synthetic mounting medium (DPX).

For anatomical investigations, the classical method of dissection was used, with the purpose of revealing organs to the digestive system. A description of each organ and its relationship to neighboring structures were made. Photographs were taken with the organs studied: teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, liver, and pancreas.

For histological and histochemical investigations, fragments of the following organs were collected: tongue, esophagus, stomach duodenum, jejunum, ileum, cecum, colon, liver and pancreas. In the case of general histological investigations, the following staining methods were used: Goldner's trichrome, and for mitochondrial evidence, we used Heidenhain iron hematoxylin staining. In the case of histochemical investigations, we used the PAS reaction to highlight neutral mucous substances and alcian blue to highlight acidic mucous substances.

In Chapter 5, entitled "Macroscopic Study of the Chinchilla Digestive System", we aimed to capture some particular aspects related to size, shape, aspect, and relationship with the neighboring structures of the components of the digestive system. For this purpose, we proposed the inventory of the anatomical aspects of the topography of the components of the digestive system at chinchilla. At the same time, we watched the surprise of some anatomical features in the organs that make up the digestive system in this species.

Chinchilla's **tongue** is a muscular organ of elongated shape. It is located on the floor of the oral cavity and occupies entirely the relatively narrow space between the dental arches so that the middle area of the tongue appears slightly torn.

Chinchilla **teeth** have long crowns and are rooted, growing continuously throughout their lives. The dental formula is 2 (I 1/1, C 0/0, P 1/1, M 3/3) = 20.

The esophagus does not have sizeable differences to consider from one end to the other and has a strong gastroesophageal sphincter.

The stomach at the chinchilla is cranially covered by the liver, and caudally it covers the spleen, which from the great curvature is attached to the large omentum to the transverse colon, thus making an indirect connection between the stomach and the transverse colon. The gastro-splenic ligament attaches the large stomach curvarture to the spleen hill. The gastro-hepatic ligament is disposed from the small curvature of the stomach to the right liver lobe. At chinchilla, the stomach is simple, undivided and has obvious longitudinal folds in the body stomach and lower in the cardia area. The fundus region of the stomach has a rounded appearance.

Chinchilla **duodenum** has a dilation called duodenal ampoule in the first portion, then a cranial flexion that continues with the downward duodenum, then a new flexure called caudal flexure, from which the duodenum continues cranial with the ascending portion.

The jejunum is the longest segmental and extends from the last duodenal portion to the ileocecal ligament.

The ileum at chinchilla is a very short segment.

The attachment elements of the small intestine segments were highlighted and described for each segment. The duodenum is supported by the meso-duodenum which also includes a part of the pancreas. The downward duodenum is attached to the mid-colon of the ascending colon with a peritoneal fold called the duodeno-colic accessory ligament. The upward duodenum is anchored by the downward colon through the duodeno-colic ligament. The jejunum is attached to the meso-jejunum in which many blood vessels are present. The ileum is anchored by cecum through ileocecal ligament.

The cecum is known to be the most bulky segment of the large intestine in rodents, but relative to body mass is significantly lower than in rabbits. The ceca at Chinchilla consist of a dilated proximal part and an elongated distal portion ending at the apex. As a mood, the cecum at Chinchilla is disposed slightly on the right side of the abdominal cavity and has three bands that delimit the haustra at the base's level and the body of the cecum.

The Colon of Chinchilla is made of three portions: ascending colon, transverse colon, descending colon. The ascending colon is disposed in the chinchilla on the right side of the abdominal cavity, attached to the ceco-colic ligament, and presents sacculation on the outside. It is formed by three distinct segments: proximal, intermediate and distal. The proximal portion starts from the cecum, passes through the ceco-colic hole, and has a lot of haustra especially in the initial part. The intermediate portion of the ascending colon is disposed in proceeding the proximal segment and as the orientation is from left to right. The distal portion of the ascending colon is extended to the right colic flexure and is formed of two segments parallel to one to another, which are united at the apical flexure. The transverse colon is relatively short and makes the connection between the ascending colon and the descending colon. It is well individualized at chinchilla. The descending colon presents, at chinchilla, numerous circumvolutions (inflections) and it continues with the rectum.

The liver of chinchilla is formed by the following lobes: the caudal lobe, the right lateral lobe, the right medial lobe, the square lobe, the left medial lobe, the left lateral lobe. The gallbladder is oval in shape and is easily visible on the visceral face, being displayed in the fossa between the right middle lobe and the right lobe of the liver, its ventral side being covered by the right lobe. The falciform ligament is relatively well developed, attaches the median lobe of the liver to the diaphragm and extends to the umbilicus. The coronary ligament is very small and the right and left triangular ligaments are also very discreet. The hepatoduodenal ligament contains two parallel bile ducts that open in the proximal duodenum.

The pancreas at chinchilla is a diffuse organ with large surface area and small thickness. From the anatomical point of view, this surface can be divided into three distinct lobes: the duodenal pancreatic lobe, the gastric pancreatic lobe, and the splenic pancreatic lobe.

Chapter 6, entitled "Microscopic Study of the Digestive System at Chinchilla", starts from the fact that in the specialty literature there are little microscopic details about the structure and ultrastructure of the digestive system components at Chinchilla, In this context, we considered it appropriate to make histological investigations of the main components of the digestive system of the chinchillas raised in captivity in our country. For this purpose, we have proposed to do a histological study of the main components of the digestive system at chinchilla, to surprise some eventual structural features of the components of the digestive system at Chinchilla raised in captivity.

The Chinchilla's **tongue** has filiform papillae all over the dorsal surface, the fungiform papillae and the foliate are in small number, and the circumvallate ones are present in the deep portion and contain gustative buds. The epithelium on the ventral face of the tongue is non-keratinized squamous stratified epithelium.

In the depth of the tongue, the muscular component predominates net, among which exist well vascularized and innervated connective tissue. The ratio between the muscular and the connective components is in the first quarter approximately 70% in favor of the muscular component, in the second quarter it reaches about 80%, in the third quarter 90% and in the last quarter, it decreases again to 60%. The muscular component is made up of a bunch of striated muscle cells with a particular orientation for each quarter. Salivary acini are only present in the fourth quarter.

The esophagus at Chinchilla is lined by a keratinized squamous layered epithelium, contains the muscularis of the mucosa formed by of smooth muscle and the muscularis itself formed by striped muscle.

The muscularis mucosae is formed by smooth muscle. The appearance of all layers of the esophageal wall is comparable in the three segments of the organ: cervical, thoracic and abdominal.

The stomach shows at Chinchilla the areas (regions) encountered also in other mammalian species, namely, cardia, fundus and pyloric, relatively well-delimited.

The cardia area at Chinchilla is placed on a relatively small surface that continues the esophageal mucosa, the passage from the esophagus mucosa to the gastric mucosa is relatively stretched. Here, the gastric mucosa that continues on the esophagus mucosa is short and gradually increases progressively over a large distance so that it reaches a thickness comparable to the one in the fundus area only at a certain distance. At Chinchilla, the glands in the cardia region are mixed with two different cell types.

The fundus area occupies most of the surface of the gastric mucosa. The gastric mucosa includes the pits that occupy about 40% of the thickness of the mucosa. There are some differences from one area to another regarding the depth of pits in the fundus of the stomach. The surface of the gastric mucosa, including the pits, is taped with a simple columnar epithelium. The area between the end of pits and muscularis mucosae is occupied by gastric glands that are slightly sinuous tubular glands. They contain two types of cells: acidophilic and basophilic. The acidophiles, also called parietal cells, synthesize precursors of hydrochloric acid and basophils, also called main cells, are responsible for the secretion of the pepsinogen.

In the pyloric area, the mucosa does not have the same thickness on the entire surface but alternates higher portions with the other ones shorter. At chinchilla, in the pyloric area, the pits are deep, occupying about 60% of the thickness of the mucosa. The cells that surround the lumen and the pits in this area are similar to those in the fundus area. The glands are numerous but somewhat less than in the fundus area. They are long tubular glands and with a lumen slightly larger than those in the fundus region. They appear to consist of two types of cells arranged in groups and not intercalated as at the fundus glands.

The gastro-duodenal junction represents the passage from the pyloric area of the stomach to the first portion of the small intestine, the duodenum. The most important structural and functional components of the gastrointestinal tract are the glandular cells of the pyloric region of the stomach and those of the Bruner glands from the duodenum.

The duodenum exhibits high villi covered by a simple columnar epithelium formed by enterocytes, among all a number of goblet cells intercalated. At Chinchilla, the intestinal mucosa from the duodenum level is not net delineated by the submucosa. The two components are very difficult to appreciate because muscularis mucosae are discontinued and represented only by rare isolated muscle cells. This aspect influents the disposition of the glands. Moreover, it seems that the two types of glands are disposed one in continuation to another, that is to say they form mixed glands that have in the upper half aspect of Lieberkühn glands and in the deeper, one aspect of the Bruner glands.

In jejunum, villi appear a little longer than in the duodenum and are covered by enterocytes that present microvilli with comparable length to the other portions of the small intestine. Through enterocytes, there are intercalated goblet cells whose number is small. The jejunum mucosa features only one type of gland, namely the Lieberkühn glands. As dimensions, these glands are short at Chinchilla. The glands at this level are made up of cells of the same type, which makes them have the same appearance over their entire length. The intestinal wall is very thin in the jejunum and the intestinal glands, in addition to being short, are relatively rare.

In the ileum the villi are somewhat taller than in the jejunum but more unequal. Among the enterocytes, there are also goblet cells, some more than in the previous segments, without reaching a very large number. The glands present at Chinchilla are relatively short and do not have a very high density. In the deep third of Lieberkühn glands in the ileum of Chinchilla there are glandular cells different from those in the rest of the glands. As disposal and shape resemble Paneth cells. The muscularis mucosae is thin but continuous.

The cecum has a thin wall at Chinchilla, having a very simple structure for this species. The mucosa of the Chinchilla cecum has no villi. The surface epithelium is formed of typical enterocytes with prismatic form equipped with microvilli at the apical pole. The epithelium clogs from place to place in the form of glands, which makes at the mucosal surface to exist similar small pits in some respects with those in the stomach level. The dispose of the glands is from these small pits to the muscularis mucosae, on which are aligned with the bottom. The presence of long villi in the three portions of the small intestine clearly demonstrates that intestinal absorption is largely in the small intestine as well as in other mammalian species. Those that are digested at the level of the cecum will absorb here, suggesting that in these animals digestion is predominantly gastrointestinal but partly and cecal.

The colon presents at Chinchilla with high folds dressed by the mucosa-coated by columnar simple epithelium that contains goblet cells in larger number than in the small intestine, but without being too many. The surface epithelium clogs from place to place in the form of medium-sized tubular glands, but obviously longer than in the small intestine. The glands are made up of cells very similar to those on the surface of the epithelium from which they were born. They are mucin-secreting cells, but both in terms of their form and tinctorial affinity of cytoplasmic, they are different from goblet cells. In other words, in the Lieberkühn glands of the Chinchilla's colon there are two different types of the mucin-secreting cells. The distance between the glands in the colon is greater than in most part of the small intestine. In this context, the lamina propria is relatively well represented in this species and cellular infiltration at its level is of medium level, with differences from one area to another. The muscularis mucosae is well represented, disposed on two planes and something thicker than at the small intestine.

Chapter 7 titled "Histochemistry of the digestive system at Chinchilla" followed the identifying mucin secretory cells. The inventory of the most well-known mucins synthesized and secreted by glandular cells in the structure of the digestive's tube organs, as well as by other less well-known, was pursued. For this purpose, we have proposed inventory of mucin-secreting cells at the level of at the segments of the digestive tube, as well as highlighting the type of mucin secreted by the cells existing in each segment of the digestive tube. We've only studied segments of the digestive system containing glandular cells.

At the **stomach** level, positive PAS elements that are present in all areas of the stomach and of comparable intensity are the surface cells of the gastric mucosa and those that line the gastric pits. The reaction is present only in the apical half of the cells, that is, the area where the mucus synthesized by these cells accumulates. The intense positive PAS reaction of the apical pole of these cells suggests that they synthesize large amounts of neutral mucosubstances. At the level of the gastric glands, there are differences from an area to another of the stomach and sometimes from one portion to the other at the same glands.

The glands in the *cardia* region exhibit a positive zonal PAS reaction in the sense that cells with mild-to-moderate PAS positive reaction occur grouped by only in certain portions of the gland and the other cells in the gland are negative PAS.

In the *fundus* region, positive PAS cells only exist at the mucosal surface and in pits, not in the fundus glands that are totally formed of negative PAS cells.

In the *pyloric* region, there are positive PAS cells also in the walls of the glands, but their number is relatively small. At this species are presented only relatively rare portions of glands containing cells with PAS-positive reaction but with weak intensity.

At the reaction with Alcian blue, the surface gastric mucosal cells, as well as those that line the gastric pits, are negative in all three areas of the stomach.

At the level of *gastric glands* situation is slightly different, at least for some of them. In the cardia area, the reaction is moderately positive but present only in a small number of glands, including here only groups of cells located in the deep portion of the glands. The glands from the fundus region do not show positive alcian cells, not even small groups or isolated cells. In the pyloric area, is noted at Chinchilla a discrete positive alcian reaction and present only at cells in the deep area of some of the glands.

In the duodenum, the goblet cells existing in the villi epithelium as well as in the Lieberkühn glands show an intense PAS positive reaction. The intensity of the reaction is medium and somewhat comparable, both at the case for cells in the villus epithelium and glandular epithelium. This aspect also suggests from the point of view of the composition of goblet cell secretion that the similarities are very large and contain neutral mucosubstances in high quantity. Regarding the cells in the Bruner glands, the situation here is different from the goblet cells, in the sense that the goblet shows a positive average PAS reaction and those in the Bruner glands are moderate PAS positive.

The reaction with alcian blue is intense alcian positive for goblet cells but also for cells in the Bruner glands, the intensity of the reaction being comparable between the two types of cells.

In the **jejunum**, at the studied species PAS reaction appears positively only for goblet cells present in the surface and glandular epithelium. The intensity of the reaction is comparable to that found in goblet cells from the duodenum level. The situation is also maintained in the reaction with Alcian blue which is also positive on caliciform cells and intensity comparable to those in the duodenum.

In the **ileum**, the histochemical reactions used in this investigation are detectable in goblet cells present in the surface and glandular epithelium, which are both PAS positive and alcian positive. The intensity of the two reactions in the goblet cells case from the Chinchilla's ileum is comparable to that found in goblet cells in the previous segments.

In **cecum**, the only cells presenting PAS positive and alcian positive reactions are the goblet cells existing here in small numbers. The intensity of the reaction is comparable to the goblet cells present in the anterior segments of the digestive tube.

In the colon, goblet cells are better represented than in the cecum, without reaching a very large number. They exhibit PAS positive and alcian positive reactions, comparable to those found in goblet cells in other segments of the digestive tube. It appears that the secretion of these glandular cells only has the role of lubricating the intestinal segments, and in this situation, there is no need for different secretion as a chemical structure and only like quantity, depending on the consistency of the intestinal content from each segment.

Particularly, at the level of the Chinchilla's colon are present in some of the Lieberkühn glands, cells with particular appearance and layout only in a portion of these glands. These cells show moderate PAS positive reaction. The intensity of the reaction is different from that of the goblet cells, which clearly suggests that they are not goblet cells, even though their appearance of cytoplasm on the topographic staining suggests that they are mucin secretory cells.

These cells are positive also at the reaction with alcian blue, and more so here the intensity of the reaction is comparable to that of goblet cells. Is it possible that the acid mucosubstances synthesized by these cells may be comparable to those synthesized by the goblet cells. This aspect, instead of simplifying things, complicates them further, in that although morphologically, these cells are completely different from the goblet ones, they functionally differ in the secretion of neutral mucosubstances, and somewhat comparable of acidic mucosubstances.

Based on the results obtained by histochemical investigations, we can state that the classical goblet cells present in a certain number in each segment of the small intestine and the large intestine are identical both morphologically and functionally. Their number is smaller than at most mammalian species (dog, cat, etc.), especially in the large intestine, where these cells are slightly larger than in the small intestine. The secretion of the Lieberkühn glands in the different segments of the intestine seems to be on the one hand similar and on the other hand different at the level of segments. We refer here to the Lieberkühn glands in the Chinchilla colon, which contain cells comparable to those in other segments or from other species, as well as cells with a particular appearance that seem to be secreting some substances that are synthesizing only here.

Chapter 8, entitled "Appreciation of general activity of hepatocyte in some morphological aspects", includes investigations into the degree of mitochondrial loading of hepatocytes. Hepatocytes represent the majority of the liver cell population

(80%) and are arranged in the form of cordons placed in intimate contact with the sinusoidal capillary.

We have not found in the specialty literature any concrete data on the degree of mitochondria loading with hepatocytes from the three areas of the liver at chinchilla. We believe that mitochondria load most closely reflect the intensity of a cell's activity. That is why we intend to evaluate the degree of mitochondrial load of the hepatocytes in the three areas of the liver lobe and to capture possible differences between the hepatocytes in each area.

The energy required to carry out all cellular activities is provided by the ATP molecules obtained at the level of mitochondria by the oxidative phosphorylation process. Depending on the intensity of cellular activities, the amount of energy used by cells is different, and the differences may sometimes be large or even very large. In order to have large or very large amounts of energy, the cells must be equipped with a corresponding number of mitochondria, in direct relation to the intensity of the activities carried out by each cell in part. We found differences related at the mitochondrial charge degree of cells in the three areas of the hepatic lobe. Cells in the 3rd zone generally have a modest mitochondrial load degree but also the smallest differences between hepatocytes from this point of view. In this context, we can state that the 3rd zone is the most homogeneous in terms of mitochondrial loading between the three areas but at the same time with the lowest loading level.

In the second area the situation is different in the sense that per total area, the degree of mitochondrial load is obviously higher than in the third zone. The difference is evident as against zone 3 and the differences between the hepatocytes of this area, which are obviously higher here. Approximately 60% of the hepatocytes present here more mitochondria, but around 40% have a similar appearance to that of the third region, which gives a particular mosaic aspect to this area. As a final estimate of the area, we can say that the hepatocytes here have a medium degree of mitochondrial loading compared to the other two areas, and the differences between hepatocytes here show the greatest differences in mitochondrial charge.

In the first zone, the mitochondrial charge level is clearly above the level of the second zone and especially of the third zone. There are also differences in this zone, but the ratio between high-loading cells and those with a lower density of mitochondria is not the same and not even comparable with the situation in the second area. Here, cells with a high density of mitochondria are net a majority, while those with average loading do not exceed 10%, and cells with a small number of mitochondria are only a few. In this context, the general appearance of the first zone is obviously more uniform than in the second zone, but there are still some differences.

Hepatocytes in all areas are considered to be comparable in terms of metabolic potential. After our investigation, it has been shown that this potential is expressed by several factors, the most important being oxygen and energy, but the age of the cell can't also be neglected. It is well known the fact that the cell does not have the same metabolic potential throughout its cell cycle.

The results obtained by us point out that the assessment of the functional activity of hepatocytes only according to the different degree of oxygenation is useful but incomplete. In order for the assessment of the metabolic capacity of the hepatocytes to be as correct and true as possible, it is necessary to take into account other factors among which the most important are the energy and age of the hepatocytes.

Chapter 9, entitled "General Conclusions", synthesizes the most important aspects, as follows:
Chinchilla has 20 teeth, a uniform caliber esophagus and an uncompartmented stomach, with obvious longitudinal folds at the level of the gastric mucosa from the stomach body area and more discreet in the cardia area.

Chinchilla's liver has six distinct lobes, namely the caudal lobe, the right side lobe, the right medial lobe, the square lobe, the left medial lobe, the left lateral lobe, and the pancreas in three lobes: the duodenal pancreas lobe, the gastric pancreatic lobe, and the splenic pancreas lobe.

Cells that lengthen the gastric mucosa and the pits exhibit intense PAS positive reaction, the glandular cells in cardia and pyloric regions show PAS positive reaction only in some cells, and those in the fundus region are negative PAS.

The Lieberkühn glands in the Chinchilla's colon have the most classical structure, but some of them have deep third mucous cells different from the goblet cells, which is why they can be considered mixed tubular glands.

The presence of positive PAS cells and positive alcian cells in the deep zone of the Lieberkühn glands of the Chinchilla colon demonstrates that they are not comparable to those of most mammalian species and can be considered as mixed tubular glands.