PHD THESIS

Morphological and histochemical study of the digestive system in Guinea pigs

SUMMARY OF THE DOCTORAL THESIS

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INTRODUCTION

The guinea pig is native to South America where it lives in a state of freedom in several countries such as Peru, Uruguay, Bolivia, Argentina, Brazil, Guyana. The species was formed and lives at high altitude, on the ridges of the Andes mountains, where conditions are harsh. Guinea pigs are forced to endure large variations in temperature and feed on slightly succulent plants in these arid areas.

The name most used both among the human population and in the specialized literature is Guinea pig. This name is erroneous because even though it physically resembles suckling piglets, the guinea pig has nothing in common with the domestic or wild pig, moreover it is not native to African Guinea as originally thought but from Guyana, a country in South America, where it still lives in a state of freedom. However, the name has been preserved and is still widely used.

Due to the exceptional anatomical and physiological peculiarities that the guinea pig has, he was quickly used as an experimental animal. The use of guinea pig for scientific purposes has taken on such a scale that it has become a veritable symbol, so that animals and even people who participate in certain tests are called "guinea pigs". The use of guinea pigs in scientific research peaked around 1960, so that their number was progressively reduced later due to the increasing use of mice and rats as experimental animals. However, the guinea pig is still much used in scientific research and thanks to the qualities it has it will also be used in the future for a long period of time that can stretch over hundreds of years or even more.

To a very large extent, guinea pigs are used as pets due to the fact that they are very gentle, clean animals and do not exude unpleasant odors.

THESIS STRUCTURE

The doctoral thesis entitled "*The morphological and histochemical study of the digestive system in guinea pigs"* comprises 150 pages and contains 58 figures. In accordance with the new methodologies for writing doctoral theses, the thesis was developed and structured in two parts.

The first part of the thesis spans 34 pages and comprises 2 chapters.

Chapter 1, entitled '*Guinea pig – general aspects*', contains aspects of taxonomic classification, guinea breeds, breeding and maintenance of guinea pigs, anatomical features, reproduction of guinea pigs, behaviour and senses in guinea pigs, the economic and social importance of guinea pigs and diseases of guinea pigs.

Chapter 2, entitled "Digestive system **"** contains general information about the components of the digestive system in the most common mammalian species that are bred in our country.

The second part of the thesis comprises 102 pages, it is structured on 8 chapters, in which are presented the working hypothesis, objectives, materials and methods, the researches of macroscopic, microscopic and histochemical morphology, on the digestive system of guinea pigs, as well as the appreciation of the secretory activity

of hepatocytes by the degree of loading with Golgi complexes. Finally, the general conclusions and aspects of originality and the innovative contributions of the thesis are presented.

OBJECTIVES OF THE WORK

-Macroscopic study of the organs that make up the digestive system in guinea pigs, in order to capture possible particular aspects;

-Microscopic study of digestive system organs in guinea pigs to identify possible adaptive histological structures due to dietary changes;

-Histochemical study of glandular structures in order to be able to assess the nature of the secretion in each cell type;

-Microscopic study of enterocytes for indirect assessment of their secretory activity by the degree of loading with Golgi complexes.

MATERIALS AND METHODS

The study was conducted on 10 corpses presented at the Zalau County Laboratory for histopathological diagnosis. The macroscopic investigations were performed using the classical method of dissection that is practiced at the Faculty of Veterinary Medicine in Cluj-Napoca. After highlighting the organs of the digestive system.

Microscopic investigations were carried out on fragments of the following organs: tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, liver and pancreas. Goldner trichrome dyes were used for general histological investigations, the Elftman method for the Golgi Apparatus, the PAS reaction for highlighting neutral mucosubstants and the alcian blue coloration for highlighting acidic mucosubstants.

RESULTS AND DISCUSSIONS

In **chapter 5** entitled " *The macroscopic study of the digestive system in guinea pigs"* was made the inventory of anatomical aspects regarding the topography of the digestive system organs in guinea pigs.

The tongue of the guinea pig is an elongated organ, arranged at the level of the floor of the oral cavity, it is of reduced width due to the fact that the dental arches are close together, and the average length is about 3.2 cm in the adult animal.

Guinea pig dentition is represented by 20 teeth with the following dental formula : I= 1/1, C= 0/0, Pm=1/1, M=3/3. All teeth (incisors, premolars, molars) have deep roots in the form of a chisel.

The esophagus of guinea pigs is divided, as in all species of mammals, into three segments: cervical, thoracic and abdominal.

The stomach, in guinea pigs, appears in the form of a postdiafragmatic transverse dilation with oblique, dorso-ventral arrangement, from left to right and flattened cranio-caudal. It presents for study two faces, two curvatures and two extremities.

The guinea pig duodenum has a short cranial portion called the duodenal ampulla that continues with the obviously longer descending loop. It undergoes a ventral inflection and continues with the ascending loop.

The guinea pig jejunum presents a series of inflections that form the jejunal loops.

The guinea pig ileum has a length equal to the length of the peritoneal fold that connects the cecum and the ileum.

The cecum occupies, caudal segmental of the abdominal cavity and shows rei portions: the ampullary portion, facing to the left, the body, arranged obliquely and the tip of the cecum, oriented slightly ventro-cranio-medially right.

The colon is divided into guinea pigs into three segments: ascending, transverse and descending. The ascending colon has, in the studied species, a tubular aspect and is connected to the great curvature of the cecum by means of a peritoneal fold, the ceco-cholic ligament. The transverse colon is quite short and appears arranged between the ascending and descending colon. The descending colon, is the segment with the largest length and from the anatomo-topographic point of view is arranged between the transverse colon and the rectum.

The rectum, in guinea pigs, is of relatively large length and has a rather small rectal ampulla.

The liver has six lobes in the guinea pig: the right lobe, the right intermediate lobe, the left lobe, the left intermediate lobe, the square lobe and the caudate lobe. Between the left intermediate lobe and the right intermediate lobe, the gallbladder is arranged.

The pancreas of the guinea pig has in its fresh state a pinkish-reddish color and consists of three portions: the body and two lobes.

Chapter 6 entitled '*Microscopic study of the digestive system in guinea pigs' aims to* obtain microscopic details on the structure of the digestive organs in guinea pigs. In order to achieve this goal, we have set ourselves the following objectives:

***-detailed histological study of the components of the digestive system in the guinea pig growing in captivity*;**

-the inventory of possible particular structural aspects at the level of some components of the digestive system in the guinea pig bred in captivity.

The tongue of guinea pigs is covered all over the surface by pavimentous stratified epithelium, intensely keratinized on the lingual papillae and the space between them, over the entire dorsal surface. The superficial muscle of the guinea pig tongue is arranged in the form of longitudinal layers that increase in thickness from the first to the third quarter, and the deep muscle consists of muscle bundles of three-dimensional orientation.

The guinea pig's esophagus consists of the segments encountered in all mammalian species, namely: cervical, thoracic and abdominal. It contains the four tunics characteristic of the digestive tract, namely mucous, submucosal, muscular and adventition. The mucosa of the cervical guinea pig esophagus consists of the three

characteristic components, that is, the epithelium, the chorion and the muscular mucosa. The epithelium is stratified pavimentos keratinized, muscular formed by striated muscles arranged in the form of layers, and from relatively lax connective tissue, very well vascularized and innervated.

The guinea pig's stomach shows a sharp transition from the esophageal mucosa to the gastric one, without the interposition of a transition zone.

The cardia region stretches over a very small area, but the thickness of the mucosa is here relatively large, getting much closer to the thickness of the mucosa in the bottom area. In this area present are glands with lumen wider than those in the fundus area.

The fundus region occupies the largest area, the gastric mucosa is here thick and occupies a little more than half of the thickness of the gastric wall. Crypts are present on the surface that occupy no more than 30% of the thickness of the bottom mucosa. The epithelium lining the mucosa to the surface, as well as the crypts, consists of prismatic cells arranged on a single row. In the depth of the mucosa there are numerous long fundic glands, arranged from the base of the crypts to the muscle of the mucosa.

The pyloric area has mucosa of uneven thickness, alternating higher portions with others somewhat less tall. Crypts here occupy about 40% of the thickness of the mucosa. The cells lining the mucosa on the surface and those at the level of the crypts have the apical pole loaded with mucus. The glands are numerous and long, and in structure they are formed by two different types of cells.

The duodenum presents in guinea pigs high villi covered by simple prismatic epithelium that also contains a small number of caliciform cells interspersed among enterocytes. In the thickness of the mucosa are present intestinal glands (Lieberkun) with the typical appearance of tubular glands, which also contain several caliciform cells. Duodenal glands (Bruner) are present in the submucosa.

In ***jejunum*** in guinea pigs, the villi are somewhat higher than in the duodenum, and the number of caliciform cells is also small. Only one type of glands are present here, namely Lieberkun glands.

In ***the ileum*** in guinea pigs, the villi are tall but more unequal than in the jejunum and the caliciform cells are somewhat more than in the previous segments. Only one type of glands are present, but they have a very particular appearance. Approximately 2/3 superior have a comparable appearance to the classical structure of the intestinal glands being formed by enterocytes and a few caliciform cells, but the deep third contains large cells with transparent cytoplasm and pyramid trunk shape.

The cecum has a thin wall in the guinea pigs and does not contain villi but crypts somewhat similar to those in the stomach. In the thickness of the mucosa are present typical intestinal glands, significantly fewer than in the ileum and arranged from the bottom of the crypts to the muscle of the mucosa. There are also caliciform cells here, but in much smaller numbers (scrapes) than in the previous segments.

The colon presents numerous and tall folds in guinea pigs, and in the axis of some of them adipose tissue is present, quite well represented. The glands are arranged from the surface of the mucosa to the muscle of the mucosa, which is thick and consisting of two layers, one thin circular and one longitudinally thicker. Many of the glands also present here two different types of cells arranged according to the manner described in the ileum.

The guinea pig liver has a structure very similar to the majority of mammalian species, being formed by polygonal lobules whose periphery does not appear very clearly.

The pancreas in guinea pigs has a structure similar to that of other mammals. The endocrine component is present in the form of Langerhans islands of various shapes and sizes, both in the exocrine component and in the interlobular connective tissue.

Chapter 7 entitled "*Histochemistry of the digestive system in guinea pigs*" aimed at the inventory of mucine secreting cells and the category of mucins secreted by each cell type. In order to be able to highlight the secretory cells of mucine, their presence and distribution in the gastrointestinal mucosa, we set ourselves the following goals:

-inventory of the presence and distribution of mucin secretory cells in the stomach and intestine in guinea pigs;

-highlighting the type of mucins secreted by the cells existing at the level of each digestive organ.

The guinea pig's stomach shows in ***the cardia*** region a positive PAS reaction to the cells of the surface epithelium of the mucosa and those that wallpaper the gastric crypts are intensely PAS+ in their apical half. As for the cardial glands, pas+ material is found only very discreetly and only in some of them.

In ***the fundus*** region, the cells on the surface of the gastric mucosa, and those lining the crypts, are intensely PAS positive. In terms of quantity, the PAS+ material is above that in the cardia region. The cells in the walls of the fundic glands in guinea pigs are PAS negative.

In ***the pyloric*** region, the situation remains with regard to cells on the surface of the mucosa and those in the crypts, where the PAS reaction is intensely positive. The cells of the pyloric glands appear positive PAS in the deep half and negative PAS in the superficial half.

In ***the cardia*** region, the cells lining the mucosa on the surface and in the crypts are negative to the reaction with alcian blue. At the level of the cardal glands there is an alcian positive reaction, but in a small number of cells and of very discreet intensity.

In the ***fundic*** region, the situation remains with regard to the negative alcian reaction of the cells lining the mucosa on the surface and in the crypts, but of those in the glands.

In ***the pyloric*** region, the situation remains with regard to the cells lining the mucosa on the surface and at the level of the crypts. A difference is found with regard to the pyloric glands where the cells in their deep half show a positive alcian reaction.

In the **duodenum** in guinea pigs, the positive PAS reaction is present both in the surface epithelium that covers the villi and the space between them and in that of the Lieberkun and Bruner glands. Enterocytes are PAS negative, and caliciform cells show intense positive PAS reaction. The situation is also preserved in the case of the Lieberkun glands. In the case of bruner glands, cells are PAS positive but the intensity of the reaction is significantly lower than in the case of caliciform cells.

In **jejunum** in guinea pigs the situation is comparable to that in the duodenum in terms of caliciform cells that also occur here intensely PAS positive, both those in the villi epithelium and in the wall of the Lieberkun glands.

In **the guinea pig ileum** there are similarities with the previous segments relating primarily to the fact that the caliciform cells present in the epithelium on the surface as well as in the walls of the Lieberkun glands, are comparable in all respects with those of the anterior segments. Particular for this intestinal segment is the fact that dcs cells are present in the deep half of the Lieberkun glands.

At **the cecum** levelthere is a small number of caliciform cells, which, however, have a positive PAS reaction of intensity comparable to those in the previous segments.

At **the level of the colon**, caliciform cells are present in both the surface and glandular epithelium. They show a positive PAS reaction comparable both to each other and to the calycidform cells existing in the segments described above.

In addition to the existing caliciform cells in the surface and glandular epithelium, there are also PAS positive DCS cells.

The reaction with alcian blue revealed particular aspects related to the activity of secretion of acid mucins.

At **the level of the duodenum** there are alcyano-positive cells in both the structure of the surface and glandular epithelium. In the surface epithelium and in the Lieberkun glands, the only positive alcian cells are the caliciform cells. In the case of bruner glands, all glandular cells show a positive alcian reaction.

In **the guinea pig jejunum**, the only positive alcian cells are the caliciform ones.

In **the guinea pig ileon**, the positive alcyano reaction of medium intensity is present in the caliciform cells regardless of their location as well as in the CELLS of dcs

In **the guinea pig's cecum**, only the calycid cells show positive alcian reaction.

In **the colony** of guinea pigs are alcian positive calycidform cells and DCS cells.

In **the guinea pig pancreas**, the caliciform cells identified in the epithelium of the interlobular excretory ducts were positive on both pas and alcian blue reactions.As for the cells in the glands annexed to the interlobular excretory ducts, most of them are negative PAS and negative alcian, and only the cells grouped in the deep portion (those with the appearance of mucus-secreting cells) appear positive to the two histochemical reactions used by us.

Chapter 8 entitled "*Indirect assessment of the secretory activity of enterocytes by the degree of loading with Golgi apparatus*" aimed at the morphological assessment of

the secretory activity of enterocytes in the guinea intestine. The proposed objectives were:

-special histological processing to highlight Golgi complexes in enterocytes of the surface and glandular epithelium;

-comparative assessment of the degree of loading with Golgi complexes of enterocytes from the surface and glandular epithelium.

The impregnation technique used by us (Elftman method) highlighted golgi complexes at the level of all enterocytes, both those on the surface of the intestinal mucosa and those in the walls of the intestinal glands.

At ***the level of the duodenum*** we compared the degree of loading with Golgi complexes of enterocytes that cover the intestinal villi, compared to those existing in the walls of the Lieberkun glands. In the case of all enterocytes we found a relatively large number of Golgi complexes with supranuclear disposition. If we compared the enterocytes on the surface with those in the Lieberkun glands, we found that their degree of load with Golgi complexes is at a very close level.

In ***the jejunum*** there are only short Lieberkun glands, and the cells in their walls contain in the cytoplasm Golgi complexes at a level comparable to that existing in the Lieberkun glands of the duodenum. If we compare the degree of load with Golgi complexes of cells in the Lieberkun glands with those on the surface of the mucosa we do not notice differences.

In ***ileum*** the situation is also comparable in terms of the degree of loading with Golgi complexes of enterocytes on the surface of the mucosa and those in the Lieberkun glands. Further, the degree of load with Golgi complexes is comparable in the three segments of the small intestine of guinea pigs.

In **the cecum** there is also a load with Golgi complexes at the level of the Lieberkun glands comparable to the situation in the segments of the small intestine, and in the enterocytes on the surface of the mucosa the load is above that of the glands. Compared to the segments of the small intestine, enterocytes on the surface of the mucosa of the guinea pig's cecum show a somewhat higher degree of load.

In the **colon,** the situation is somewhat comparable to that in the cecum, since here, too, the enterocytes on the surface show a degree of load with Golgi comnplexes, higher than those in the walls of the Lieberkun glands.

Chapter 9 entitled '*General conclusions*' contains a summary of the most important aspects captured by the investigations carried out, as follows:

The tongue of guinea pigs is covered all over the surface by keratinized pavimentous stratified epithelium that also continues in the esophagus, the muscles of the esophagus are formed by striated muscles in both the prediafragmatic and postdiafragmatic portions, and the submucosa does not contain glands.

The cardia region occupies a very small area in the guinea pig's stomach and the gastric mucosa represents 50% of the wall thickness and has on the surface crypts that

occupy about 30% of the thickness of the mucous membrane in the cardia region, 25% in the fundus region and 40% in the pyloric region.

Duodenal submucosal glands appear formed in guinea pigs only from mucous cells and are present in large numbers along the entire length of the duodenum, but they extend over a certain distance and into the jejunum where their number is significantly smaller and the distance between them much greater.

The Lieberkun glands are relatively short and not very often in the duodenum and jejunum, but somewhat longer in the ileum and colon where they appear formed in the upper half of enterocytes and caliciform cells, and in the deep half of particular cells with typical appearance of mucin secretory cells, called DCS cells.

The guinea pig pancreas contains langerhans islands both intralobular and interlobular, and the interlobular excretory ducts have numerous typical caliciform cells and slightly sinuous tubular glands, formed by prismatic cells and DCS cells, grouped characteristically.

Taking into account that neutral mucine secretory cells are found in all regions of the stomach, and acidic mucine secretory cells only in the pyloric glands and to a small extent in the cardial ones, we can say that the mucous secretion of the stomach is mainly represented by neutral mucins.

The cells of the duodenal submucosal glands are positive to both the PAS reaction and the one with alcian blue, which proves that they synthesize both categories of mucins, but in moderate amounts, the intensity of the secretion being below that of the caliciform cells.

Regardless of their location, the caliciform cells are intensely positive to the PAS reaction and of medium intensity to the one with alcian blue, which proves that they synthesize both neutral mucines and acid mucines, and quantitatively the neutral ones surpass the acidic ones, so that their secretion is predominantly neutral.

Regardless of their location, DCS cells are positive to both histochemical reactions, which proves that they secrete both types of mucins, and the intensity of the reaction is higher to the reaction with alcian blue and weaker on the PAS reaction, so their secretion is predominantly acidic.

It is possible that the predominantly neutral secretion of the caliciform cells from the pancreatic ducts ensures the protection and some lubrication of the ductal epithelium, and the predominantly acidic secretion of the cells of the ductal glands ensures the maintenance of the pancreatic enzymes in the inactive state until they reach the intestine.

The investigation carried out by us revealed a relatively high degree of loading with Golgi complexes in the cytoplasm of enterocytes, both those in the surface epithelium and those in the Lieberkuhn glands, in all segments of the small intestine and large intestine.

The degree of golgi complex load on enterocytes on the surface of the intestinal mucosa is somewhat higher in the large intestine than in the small intestine, which

suggests that the secretory activity of enterocytes in the large intestine is somewhat higher than those in the small intestine.

At a general comparative assessment we can say that the absorption activity surpasses to some extent that of secretion in the duodenum, jejunum and ileum, it is carried out at a somewhat comparable level in the cecum, and in the colon the activity of secretion clearly exceeds that of absorption.

In this situation, if we make an overall assessment of the intestine, it is quite possible that the secretory activity of enterocytes also exceeds that of absorption, although this is predominant in the segments of the small intestine.

The presence of DCS cells in the guinea pig ileum suggests that their secretion product participates in the development of phenomena common to some extent to the terminal portion of the small and large intestines, probably preparing food for a cecal digestion as easy as possible.

It is possible that the secretions of the Lieberkun glands also participate in the regulation of the consistency of the intestinal contents according to the needs, and in pathological situations the massive fluid loss is made primarily by amplifying the glandular secretion and only partially by other mechanisms

Chapter 10 entitled "*The originality and innovative contributions of te*zei" points out the most important results obtained in this thesis.

The anatomical studies carried out complete the few thorough studies carried out on the digestive system of guinea pigs, existing in the specialized literature.

Histological studies have revealed some aspects less known, or even unreported so far, on the digestive system of guinea pigs. Among the more important aspects we mention:

The tongue of the guinea pig has keratinized pavimentous stratified epithelium over the entire dorsal surface.

The tongue of guinea pigs does not show glandular acini in the first three quarters, but only in the fourth quarter, serous acini and mucous.

None of the segments of the esophagus of guinea pigs contain glands.

The musculature of the esophagus is formed by striated muscles, along the entire length of the organ.

The cardia region occupies a very small area in guinea pigs and has crypts of comparable length to those of the bottom region.

The gastric mucosa of the *pyloric region* of the guinea pig stomach does not have the same thickness all over the surface, but alternates high portions with shorter ones.

Bruner glands extend to guinea pigs and into the initial portion of the jejunum

The Lieberkun glands are short in the jejunum, media in the ileum and in the large intestine.

We first reported the existence of DCS cells in the Lieberkun glands in the guinea pig ileum.

The Lieberkun glands in the guinea pig colon contain DCS cells in greater numbers than in the ileum.

In the walls of the interlobular excretory ducts in the pancreas there are calycidform cells and tubulo-acinar glands.

Histochemical studies have highlighted some functional aspects, among which we mention:

The cells lining the gastric mucosa synthesize only neutral mucines.

The glands in the cardia region synthesize small amounts of neutral and acidic mucines.

Glands in the fundus region do not synthesize mucines.

About 50% of the cells of the pyloric glands synthesize neutral mucins and acidic mucines.

Regardless of their location, caliciform cells synthesize large amounts of neutral mucines and moderate amounts of acidic mucins.

The Bruner glands secrete moderate amounts of neutral and acidic mucines in guinea pigs.

Regardless of their location, DCS cells synthesize both neutral and acidic mucines, predominantly acidic ones.

Studies on the secretory activity of enterocytes have highlighted the following aspects:

Secretory activity is comparable to enterocytes in the surface epithelium and those in the glands.

The intensity of the secretion is marked and comparable in enterocytes of all segments of the small intestine and the large intestine.

Overall the intestine it is quite possible that the secretory activity will outdo that of absorption.