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Original research paper

MORPHOMETRICAL ANALYSIS OF GASTRIN CELLS IN THE GASTRIC MUCOSA OF THREE-WEEK-OLD PIGS (*Sus scrofa domesticus*) AND A COMPARISON WITH OTHER GASTRIC ENTEROENDOCRINE CELLS

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Summary: The number and localisation of enteroendocrine cells from different parts of the gastric mucosa of three-week-old pigs were analysed. The basic method for the visualisation of enteroendocrine cells was immunohistochemistry and the results were compared to those derived through both the toluidine-blue staining for enteroendocrine cells and Grimelius silver staining methods. The greatest number of gastrin cells was in the pyloric mucosa with 124.66 ± 4.54 gastrin cells per mm^2 in the antrum and 140.32 ± 5.30 per mm^2 in the pyloric canal. The highest density of gastrin cells in relation to the thickness of the gastric mucosa was in the middle third of the mucosa with approximately 50 % of all gastrin cells. The remaining gastrin cells were evenly distributed in the upper and lower thirds of the mucosa. Cutaneous mucosa was negative for gastrin and other enteroendocrine cells. We found that the area of the small curvature, which has cutaneous and pyloric types of mucosa, had an average of 29.21 ± 2.97 gastrin cells per mm^2 and that the areas with cardial and fundic mucosa had lower averages of 0.61 ± 0.14 cells per mm^2 and 1.14 ± 0.11 gastrin cells per mm^2, respectively. Morphometric analyses of the serotonin and somatostatin cells from the pyloric area, where there was the highest density of gastric cells, were performed. The numbers of both types of cells were much lower compared to the gastrin cells, with 17.7 ± 2.30 serotonin cells and 50.7 ± 2.76 somatostatin cells per mm^2 of mucosa. The results of the silver staining and the toluidine-blue staining only partially correlated with the results of the immunohistochemical reaction. While many more cells reacted to the Grimelius silver staining (153.5 ± 0.32 cells per mm^2) than to the toluidine-blue staining (51.15 ± 2.20 cells per mm^2), there were still considerably less of them in comparison with the number of cells that reacted immunohistochemically.

Key words: veterinary medicine; stomach; enteroendocrine cells; gastrin; somatostatin; serotonin; immunohistochemistry; Grimelius silver staining; toluidin blue; pig

Introduction

There are four distinct regions of gastric mucosa in pigs: the oesophageal, or proventricular part (*pars oesophagealis s. proventricularis),* which is located at the gastric entrance and has cutaneous mucosa, and three parts that have glandular mucosa (*pars glandularis*). The anterior third of glandular mucosa, which is located in the anterior part of ventricular body between the oesophageal part and the *genu* ventriculi and includes the gastric diverticulum, has cardial mucosa. The second part is behind the genu and it contains fundic mucosa with fundic glands, and the third area is the caudal part of the stomach, which has pyloric mucosa and begins at the larger antrum and continues into the pyloric canal that contains numerous sero-mucosal glands in the propria (1). Enteroendocrine cells can be found scattered among the epithelial mucosal cells in all parts of the gastric mucosa (2). They belong to the diffuse endocrine system or the APUD system (amino precursor uptake and decarboxylation). In this sys-

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tem byogene amines and peptides are synthesised – neurohormones and neurotransmitters, suggesting their relation to neurones (2, 3). There are 6 types of enteroendocrine cells in the stomach: A, G, D, EC, P and ECL cells. The A cells synthesise gastro-glucagon, the G cells (argyrophil cells) produce gastrin, D cells produce somatostatin and the EC cells (enterochromaffin or argentaffin cells) produce 5-hydroxytryptamine (5-HT). The P cells produce both prostaglandin and motilin and the ECL cells (enterochromaffin-like cells), which can be visualised with the Grimelius silver staining, produce histamine (4, 5, 6).

Gastrin is a heptadecapeptide with 17 amino acids produced by gastrin cells (G cells). Gastrin cells are located within the gastric epithelium and in humans, where they have a characteristically conical or oval form with microvilli on their surface (7), the highest concentrations are found in the antrum. Their ultrastructure depends on their activity status (8) and they contain three types of granules (6, 9, 10). Gastrin molecules are synthesised from precursor molecules (10). There are two chemical forms of gastrin, gastrin I and gastrin II (7), and several biologically active forms: large gastrin G34, small gastrin G17, mini gastrin G14 and component I (11). All forms have at C-end of the molecule tetragastrin, a prolongation molecule, which is also biologically active (2). Gastrin has an exocrine function as it secrets into the gastric lumen, it has also an endocrine function and influences distant tissues by secreting into the blood, and as it regulates the activities of neighbouring cells, it also has a paracrine effect (2). Gastrin is not only important for the normal functioning of the stomach, but also for the normal functioning of other parts of the digestive system – the duodenum, pancreas, liver. In humans it has been shown to stimulate the excretion of gastric acid and pepsin, the growth of gastric mucosa, the secretion of pancreatic enzymes and the secretion of water and electrolytes in the stomach, pancreas, liver and Brunner glands. It increases the absorption of water and electrolytes from the small intestine, stimulates contractions of the stomach and gall bladder and relaxes the muscles of the pyloric and the ileocaecal sphincter. It also stimulates the secretion of insulin, acetylcholine, somatostatin, pancreatic polypeptide and calcitonin as well as affecting the proliferation of ECL cells (2, 12, 13, 14, 15).

There is already a lot of published data regarding gastrin cells of different animal species. Three types of gastric cells have been described in newborn rats, according to their level of differentiation - primitive, transitional and typical gastrin cells (9). However, very little data is available that describes at what age they appear and if there are any age-dependent variations in their numbers and localisation within the gastric mucosa and physiological characteristics of the animal species at certain ages. We found very few references relating to the gastrin cells of the pig, and even fewer pertaining to the different pre- and postnatal developmental stages and to the adaptation to the changes in the feeding practices after birth and weaning. The majority of gastrin in the stomach of pigs is small gastrin 17 (16) and there are equal quantities of gastrin 17 and its precursor, gastrin 34 (17). The secretion of gastrin depends of several factors: the composition of food, the quantities of releasing and inhibiting peptides (GRP, GIP) in the circulation, nervous factors (vagus), and paracrine and endocrine factors (prostaglandin, somatostatin, histamine etc.) (18, 19). In this study we analysed the gastric mucosa and gastrin cells of three-week-old pigs. At this stage the pigs are weaned and their gastric mucosa has to quickly adapt to new types of food. It is also a period when piglets experience frequent gastrointestinal disorders. For comparison we also analysed the localisation and distribution of the somatostatin and serotonin cells, both of which are involved in controlling gastrin secretion.

Material and methods

Materials

Ten weaned, three-week-old, farm pigs were killed and their stomachs, which were immediately removed from their abdominal cavity, were opened along the major curvature. The stomach content was removed and samples were taken from six parts of the gastric wall: from the cardia, fornix and gastric body, and from the antrum, pyloric canal and the minor curvature. The tissue was then fixed in buffered formalin for approximately 24 hours before being embedded in paraffin following standard laboratory procedures. The paraffin blocks were then cut into 7µm thick sections.

Histology

One tissue section from each of the gastric regions was stained with haematoxylin and eosin for the histological examinations.

Immunohistochemistry

The immunohistochemistry was performed using the PAP method (peroxidase - anti-peroxidase) described by Sternberger (20). We applied a 1:1500 dilution of polyclonal rabbit antihuman gastrin-17 as primary antibodies (DAKO, cat. no. A568) to slides that were then incubated in a humidified chamber overnight at 4 °C. These antibodies also react with gastrin-34. The slides were then treated with a 1:50 dilution of goat anti-rabbit immunoglobulins (Amersham) prior to a final treatment with a 1:50 dilution of PAP complex (Sigma, cat. no P1291). All the dilutions were made with a 2.5 % solution of bovine serum albumin (BSA) in a buffered phosphate saline. DAB (3'–diaminobenzidin tetrahydrochloride, Sigma, cat. no. D5905) was used as a substrate and the tissue sections were counterstained with either haematoxylin or 1 % methyl green, dehydrated and mounted with synthetic resin. After we had completed the morphometric analyses of the PAP tissue sections the DAKO LSAB®2 System, HRP product (Dako, cat. no. K0675) became available on the market. By using it in accordance with the manufacturer's instructions we were able to assess the quality of the product by making a comparison of the immunohistochemical reactions of the PAP method and this new system.

Grimelius silver staining (21)

We used the Grimelius silver stain to determine the numbers of gastrin cells and compared these results with those of the PAP method. This histochemical method was also used to determine the population of all the enteroendocrine cells in the stomach that react with this type of silver staining.

Toluidine-blue staining for enteroendocrine cells (22)

The toluidine-blue staining method, which is adjusted for the detection of enteroendocrine cells, supposedly enables a partial differentiation of these cells without the use of immunohistochemistry. Cells containing gastrin should stain in a metachromatic manner and other enterochromaffin cells in an orthochromatic manner, e.g. cells containing serotonin, after a pre-treatment in hot hydrochloric acid.

Treatment of serial tissue sections using all the methods and a double-PAP procedure for the localisation of gastrin and other enteroendocrine cells

Serial tissue sections were prepared using only the samples from the pyloric part of the stomach. One tissue section was treated with toluidine blue, on another tissue section we demonstrated gastrin cells using the PAP method and a third one was silver stained following the Grimelius silver-staining procedure. A fourth tissue section was immunohistochemically treated for a reaction to serotonin using the PAP method and a 1:1000 dilution of polyclonal rabbit anti-serotonin antibodies (Inc Chemical Credential). A fifth tissue section was used for a double-immunohistochemical reaction - the first layer for gastrin and the second layer for somatostatin (Dako, cat. no. A 566, diluted 1:1500). A sixth tissue section was used for a double-immunostaining procedure to determine serotonin (first layer) using a nickel-ammonium-sulphate-enhanced DAB substrate (23) that gives either a dark brown or a black insoluble-reaction product; and somatostatin (second layer) using a DAB substrate that gives an insoluble brown-reaction product. We also localised somatostatin-immunoreactive cells on another tissue section. As a comparative measure, the LSAB technique was also used later to detect serotonin, somatostatin and gastrin.

The double-immunohistochemical reactions were achieved using the steps of the PAP method with the application of the first primary antibody and the incubation in the first substrate. However, instead of being dehydrated, the tissue sections were incubated with a second primary antibody in a humidified chamber overnight at 4 °C. The next day the procedure was concluded with repeating of all the consecutive steps of the PAP method that are usual after incubation with primary antibodies. The only change was the use of the different substrate. During the optimisation of the procedure we tested different combinations of antibodies, as this was found to be an impor-

Table 1: Mean values (± SE) of the thickness of gastric mucosa in different parts of the stomach of a three weeks old pig and mean number of gastin cells per mm^2 (± SE) of mucosa

Area	Mucosal thickness in µm ± SE per mm^2 of mucosa	Mean number (± SE) of gastrin cells
1	243,50 ± 0,81	0,00 ± 0,00
2	280,86 ± 1,04	0,61 ± 0,14
3	501,19 ± 1,33	1,14 ± 0,11
4	329,96 ± 1,38	124,66 ± 4,54
5	410,05 ± 0,94	140,32 ± 5,30
6	313,11 ± 1,12	29,21 ± 2,97

Legend:
1 - area with cutaneous mucosa (oesophageal part, cardia)
2 - area with cardial mucosa (fornix)
3 - area with fundic mucosa (gastric body)
4 - area with pyloric mucosa (antrum)
5 - area with pyloric mucosa (pyloric canal)
6 - area with cutaneous and pyloric mucosa (small curvature)

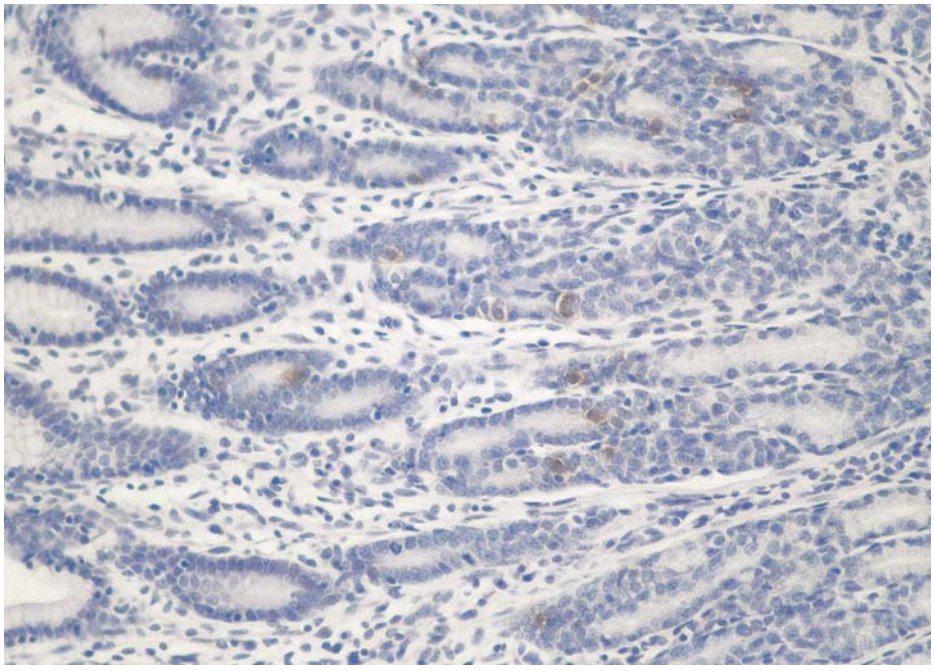

Figure 1: Cardial mucosa of a three-week-old pig, a few gastric cells. PAP, DAB, counterstained with haematoxylin, x 20

tant factor in the quality of the reaction. We achieved the best results using the aforementioned combination.

Determination of the thickness of the gastric mucosa

The average thickness of the gastric mucosa samples from the five pigs was measured using an ocular micrometer at a magnification of 100x. Ten measurements, from five locations on two consecutive tissue sections, were taken and the distances between the units on the ocular micrometer were calculated with an object micrometer.

Determination of the number of gastrin cells

The number of gastric cells was determined with a light microscope as were all the gastrin-positive cells on two tissue sections from each of the selected gastric regions. We counted the positive cells across the ten visual fields of each tissue section, i.e. 20 visual fields from each gastric

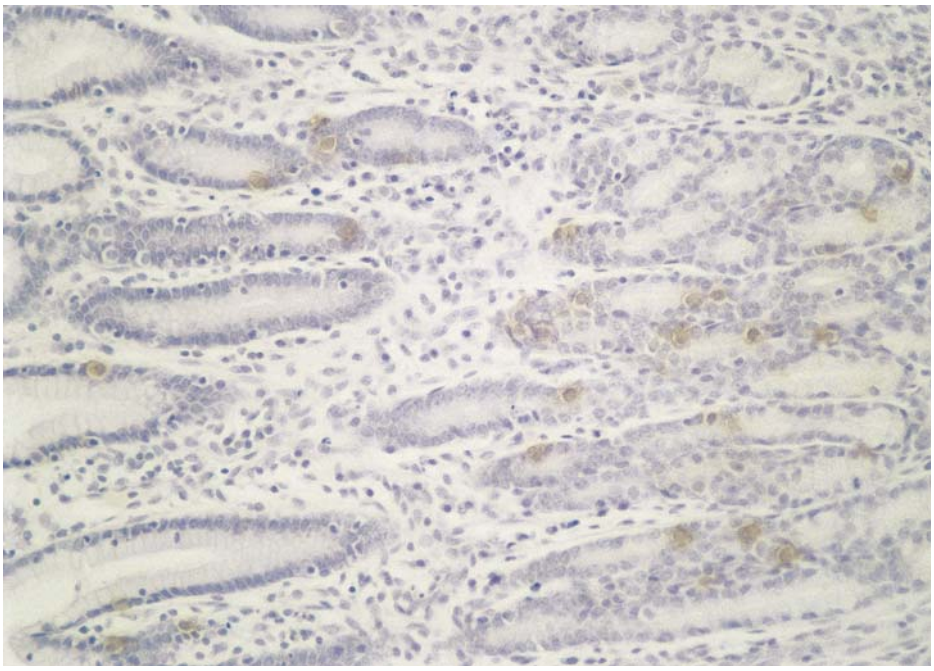

Figure 2: Cardial mucosa of a three-week-old pig, a few gastrin cells but more numerous than in figure 1 - comparative immunostaining to the PAP method (figure 1); DAKO LSAB®2 System, HRP; DAB, counterstained with haematoxylin, x 20

region. A visual field was determined as the height of gastric mucosa by the width of the field of view at a magnification of 200x. At that magnification the visual field was 530 μm wide. The area of a visual field (VFA) was calculated with the formula:

VFA = height of the mucosa x 530 (24)

The number of cells was then calculated per mm^2 of mucosa.

Statistics

The results were statistically evaluated using the Batch System software programme and evaluated with an analysis of variance. The calculations were made with a probability of 95 % (P = 0.05).

Results and discussion

A few references have been published in which the authors discuss the distribution of gastrin cells in the stomach of the pig (25) and their physiological significance at different ages (26, 27, 28). At three weeks the growth rate of a pig's stomach decreases in comparison with that of its body, which coincides with the adaptation of the gastric mucosa to the effects of active peptides, as was reported in a study on the effects of pentagastrin (29). It has also been reported that at the same age the speed of gastrin degradation in the gastric and intestinal lumen is higher in comparison with newborn and adult pigs, although it is not quite clear why (26).

In the samples of the gastric mucosa of three-week-old pigs used in our study, the thickness of the mucosa from the different regions of the stomach varied significantly, except between the antral mucosa and the mucosa from the small curvature (average thickness 329.96 ± 1.38 μm and 313.11 ± 1.12 μm, respectively). The average thickness of cutaneous mucosa was 243.50 ± 0.81 μm, of the cardial mucosa 280.86 ± 1.04 μm and the fundic mucosa 501.19 ± 1.33 μm. The average thickness of the mucosa in the pyloric canal was 410.05 ± 0.94 μm, which was significantly more than it was in the antrum, which was 313.11 ± 1.12 μm thick (Table 1).

The Grimelius silver staining revealed enteroendocrine cells in all parts of gastric mucosa, except in the areas with cutaneous mucosa. That correlated with both the immunohistochemical results and those of the toluidine-blue staining. Based on their staining properties, two types of cells were found, which could be representative of two types of enteroendocrine cells. One type of cells gave a light-brown reaction product with the silver staining while the others were dark brown. The highest concentrations of positive cells revealed by the Grimelius silver staining method were in the fundic mucosa, the antral mucosa and in the mucosa of the pyloric canal.

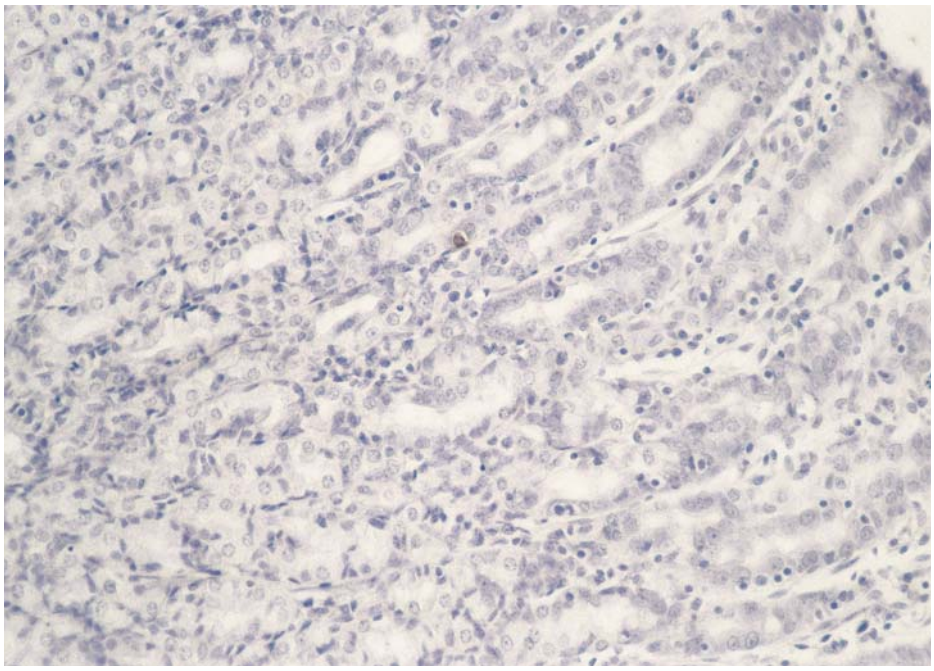

Figure 3: Fundic mucosa of a three-week-old pig, only one cell positive for gastrin; PAP, DAB, counterstained with haematoxylin, x 20

The cells were distributed throughout the mucosa with the highest concentrations being at the base of the glands with an intra-epithelial localisation.

The toluidine-blue stain reacted with cells in all areas of the gastric mucosa, with the exception of the cutaneous mucosa. The cells stained either orthochromatically or metachromatically. While the orthochromatic cells were localised among epithelial cells (intra-epithelial localisation), similar to the silver-stained cells, the metachromatic cells were extra-epithelial. As with the silver-stained cells, the greatest number of orthochromatic cells was in the mucosa of the fundus and the pyloric canal, and the least in the cardial mucosa. In all the regions, they were evenly distributed from the base to the luminal part of mucosa. Metachromatic cells were also found in all parts of gastric mucosa, with the exception of the cutaneous mucosa. Based on the localisation of the orthochromatic cells we believe that they are not gastrin cells as there was a very low number of immunohistochemically-positive gastrin cells in the fundic mucosa.

Gastrin cells revealed immunohistochemically were found in all parts of the gastric mucosa, except in the oesophageal part with cutaneous mucosa. However, there was great variation between the quantities determined in the different parts of the stomach: from just a few positive cells in the cardial and fundic mucosa to the large number of positive cells found in the pyloric canal. The highest concentration of gastrin cells was in the mid-third of the mucosa and only partly correlated with the localisation and number of cells revealed with the non-specific methods, Grimelius silver staining and the toluidine-blue staining. The intensity of the positive immunohistochemical reactions varied from light to dark brown. Although similar distributions of gastrin and other enteroendocrine cells were detected by both immunohistochemical methods, the PAP and the DAKO LSAB®2 System, it was subjectively assessed that the latter revealed more positive cells than the former (Figures 1 and 2). The distribution of gastrin cells in the gastric mucosa of the three-week-old pigs was similar to that determined by Bussolati (25) using immunofluorescence. However, as we do not know the ages of the pigs used in his study we cannot say that these results can be applied to pigs of all ages.

The distribution of the other types of enteroendocrine cells in parts of the stomach was distinctly different from the distribution of gastrin cells, with the exception of the cutaneous mucosa, which was completely negative for all enteroendocrine cells. In the mucosa of the pyloric canal we found a small number of serotonin-positive cells and unlike the gastrin cells, which were concentrated in the middle third of the mucosa, the serotonin-positive cells were evenly dispersed throughout the mucosa. In the tissue sections with double-immunohistochemical reactions, the

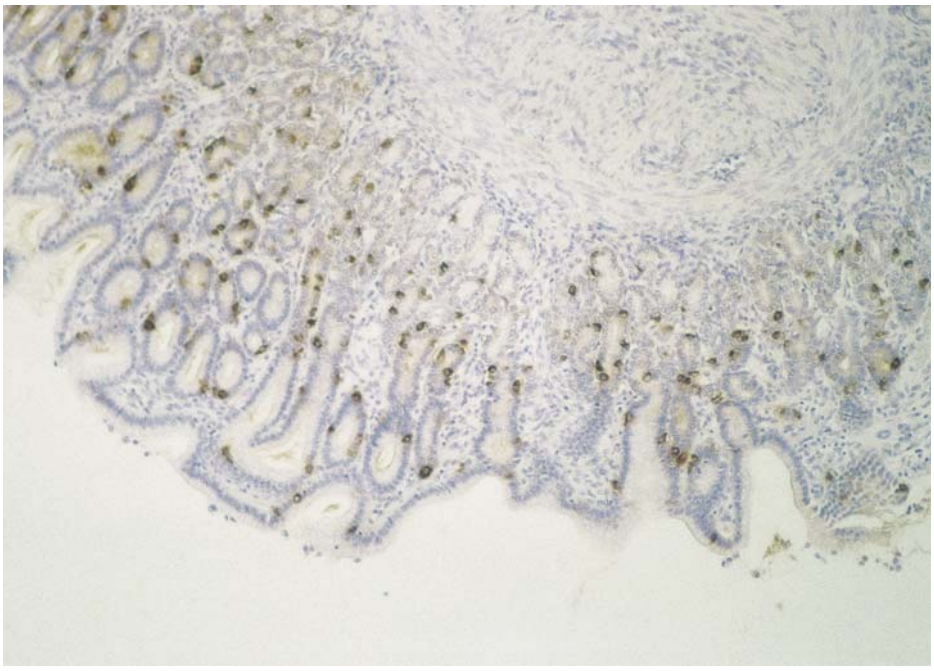

Figure 4: Pyloric mucosa of a three-week-old pig near the transition to duodenal mucosa. Numerous gastrin cells (DAKO LSAB®2 System, HRP; DAB, counterstained with haematoxylin, x 40)

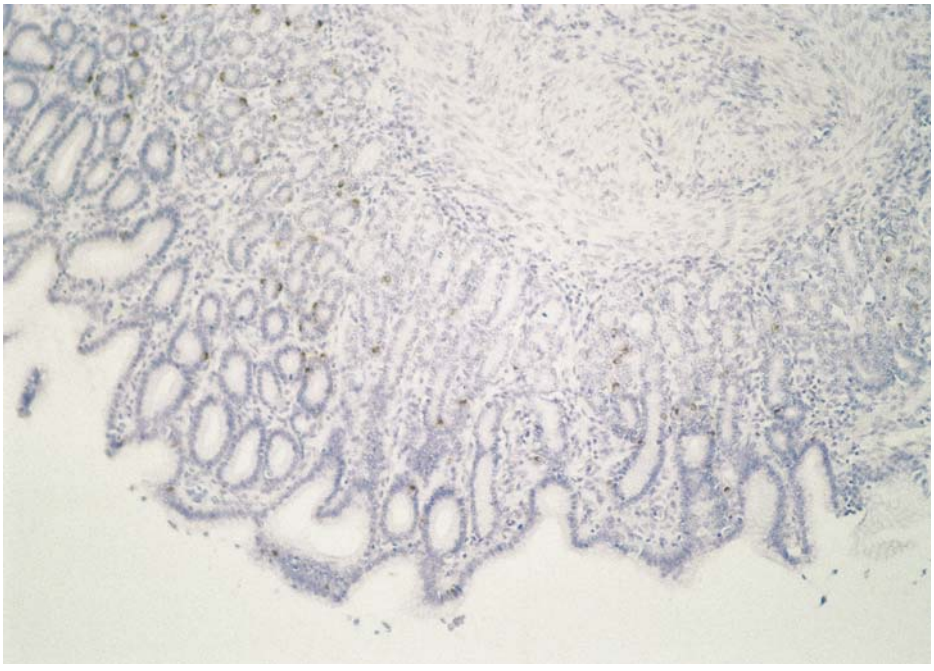

Figure 5: Pyloric mucosa of a three-week-old pig near the transition to duodenal mucosa - cells positive for somatostatin. DAKO LSAB®2 System, HRP; DAB, counterstained with haematoxylin, x 40

combinations of somatostatin with either gastrin or serotonin, we found both light and dark brown cells. This was similar to the variability in intensity of the gastrin-cell reaction product and could, in our opinion, be representative of different forms of the gastrin molecule.

The number of gastrin cells in the cardiac and fundic mucosa were statistically insignificant, averaging 0.61 ± 0.14 cells per mm^2 and 1.14 ± 0.11 cells per mm^2, respectively (Figures 1, 2 and 3). A subjective assessment of the numbers of gastrin cells revealed by both the PAP and DAKO LSAB®2 System immunohistochemical methods and a comparison of them indicated that the number of positive cells revealed by the latter was somewhat higher. This is entirely understandable, as it is much more sensitive of the two systems (Figures 1 and 2). The number of gastrin cells was significantly higher in the pyloric mucosa, both in the antrum and the canal, and

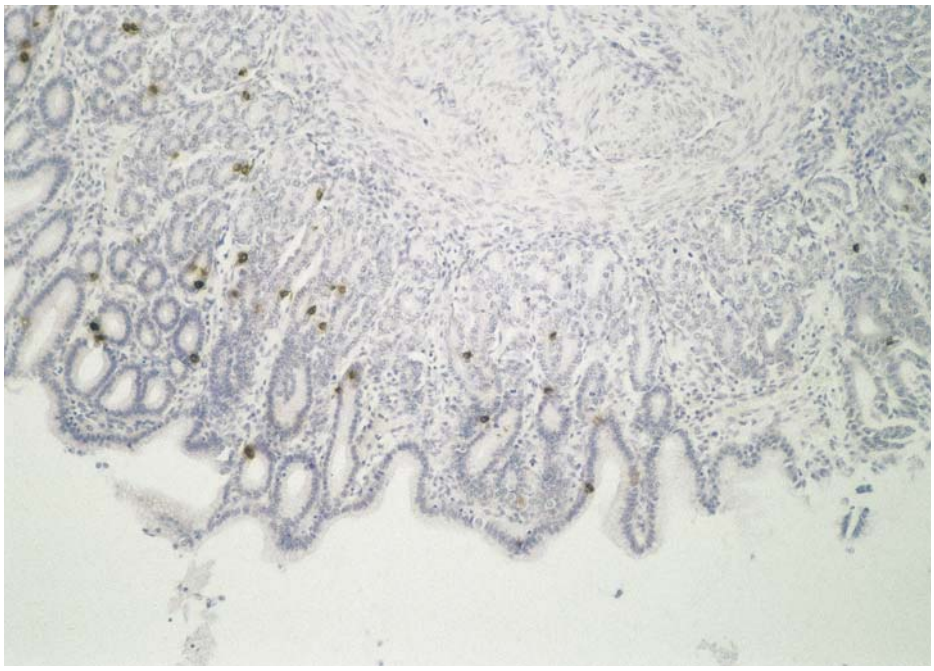

Figure 6: Pyloric mucosa of a three-week-old pig near the transition to duodenal mucosa - cells positive for serotonin. DAKO LSAB®2 System, HRP; DAB, counterstained with haematoxylin, x 40

Table 2: Mean values (± SE) for the number of cells, positive for gastrin, serotonin or somatostatin per unit of gastric mucosa (visual field) in pyloric canal of three weeks old pig, counted on consecutive serial sections, stained with toluidin blue (1), in Grimelius silver staining (3) and immunohistochemical reaction (IHC) for gastrin (2), serotonin (4) and somatostatin (5)

Number of serial section	Method / reaction for	Number of cells per mm² of mucosa ± SE
1	toluidin blue	51,15 ± 2,30
2	gastrin (IHC)	141,94 ± 5,53
3	Grimelius silver staining	153,5 ± 0,32
4	serotonin (IHC)	17,7 ± 2,30
5	somatostatin (IHC)	50,7 ± 2,76

in the area of the small curvature where cutaneous mucosa merges with the pyloric types of mucosa. The average values for the gastrin cells in these areas differed significantly. In the antral mucosa the average number of cells was 124.66 ± 4.54 per mm², whereas the average number of gastrin cells in the pyloric canal was 140.32 ± 5.30 cells per mm² and in the mucosa of the small curvature the average was 29.21 ± 2.97 cells per mm² (Table 1).

The highest concentration of gastrin cells was in the middle third of the pyloric mucosa, where the necks of the glands and the deep parts of gastric pits are located. The number of gastric cells in this part of the antral mucosa averaged 89.31 ± 7±.58 cells per mm² and 214.30 ± 8.61 cells per mm² in the mucosa of the canal. In relative terms that meant that 28.08 % of the gastrin cells in the antrum and 26.21 % of the gastrin cells in the canal were located in the basal part of the pyloric mucosa. The middle third of mucosa contained 50.62 % of all the gastrin cells in the antrum and 50.67 % of the gastrin cells in the canal; and the remaining 21.30 % of cells in the antral mucosa and 23.15 % of gastrin cells in the canal where located in the luminal third. This distribution pattern dissipated towards the duodenum where the cells were evenly distributed throughout all parts of the mucosa (Figure 4). In this area we also found cells that were immunoreactive to serotonin and somatostatin, which were evenly distributed among the gastrin cells (Figures 5 and 6).

With a few exceptions the numbers of enteroendocrine cells revealed by the different staining methods used on the serial tissue sections of pyloric mucosa differed significantly. The exceptions were the numbers of serotonin-immunoreactive cells and the number of

orthochromatic cells revealed by the toluidine-blue staining, and between the numbers of metachromatic cells in the toluidine-blue staining and in most, but not all, of the number of dark-brown cells revealed by the silver staining (Table 2).

With a comparison of the pictures taken from the serial sections it became obvious that the dark cells revealed by the silver staining were the same as the serotonin-immuno-positive cells. We were unable to confirm the same for the light-brown silver-stained cells and the gastrin cells. Orthochromatic cells were the same in both the toluidine-blue and the silver-stained cells.

The results of the morphometric analysis of the distribution of gastrin cells in the gastric mucosa of the three-week-old pigs and the comparison with the localisation and distribution of other enteroendocrine cells of the gastric mucosa added new information to the existing body of data (25). The results could also be compared with data from some publications discussing the physiological role of gastrin in the postnatal development of gastric mucosa in pigs (25, 26, 27, 28, 29). They also provide an insight into the comparitive sensitivities of some of the older non-specific methods for demonstrating enteroendocrine cells (Grimelius silver staining, toluidine blue for enterochromaffin cells) and the more specific immunohistochemical methods. The results of this study could provide a strong basis for further studies of the physiological and pathological processes of the digestive tract of the pig, given by the intensive postnatal morphological and functional changes in the digestive tract of this animal species. These are controlled and influenced by many factors (30) that could negatively affect the development of some or most of the segments of the digestive tract and enable conditions that are suitable for the development of pathological lesions in the stomach and intestines.

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MORFOMETRIČNA ANALIZA GASTRINSKIH CELIC V ŽELODČNI SLUZNICI TRI TEDNE STARIH PRAŠIČEV *(Sus scrofa domesticus)* TER PRIMERJAVA Z NEKATERIMI DRUGIMI ENTEROENDOKRINIMI CELICAMI V ŽELODCU

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Povzetek: V sluznici želodcev tri tedne starih prašičev smo določali razporeditev in število enteroendokrinih celic v posameznih delih želodca. Izhodiščna za prikaz enteroendokrinih celic je bila imunohistokemična metoda, primerjalno pa smo enteroendokrine celice barvali s toluidinskim modrilom za tovrstne celice in jih srebrili po Grimeliusu. Največ gastrinskih celic smo ugotovili v vratarjevi sluznici, v antrumu 124,66 ± 4,54 gastrinske celice na mm^2 in v kanalu 140,32 ± 5,30 na mm^2. Največja gostota gastrinskih celic, glede na višino sluznice, je bila v srednji tretjini, kjer je bilo približno 50 % vseh tovrstnih celic, preostale pa so bile dokaj enakomerno razporejene v zgornji in spodnji tretjini sluznice. Na področjih s kutano sluznico ni bilo niti gastrinskih niti drugih enteroendokrinih celic. Na mali krivini želodca, kjer se prekrivajo področja brezžlezne in vratarjeve sluznice, je bilo v povprečju 29,21 ± 2,97 gastrinske celice na mm^2, na področjih s kardialno sluznico in sluznico pravih želodčnih ali fundusnih žlez pa je bilo njihovo število še manjše: 0,61 ± 0,14 celice na mm^2 kardialne sluznice ter povprečno 1,14 ± 0,11 gastrinske celice na mm^2 fundusne sluznice. Na področju vratarja, kjer je največ gastrinskih celic, smo morfometrično določili tudi število serotoninskih in somatostatinskih celic. Obeh vrst celic je bilo v sluznici tega področja značilno manj kot gastrinskih celic, in sicer serotoninskih 17,7 ± 2,30 na mm^2 sluznice ter somatostatinskih 50,7 ± 2,76. Rezultati srebrenja in barvanja s toluidinskim modrilom so se le deloma ujemali z rezultati imunohistokemične reakcije. Celic, ki so se v vratarjevi sluznici pobarvale s toluidinskim modrilom, je bilo bistveno manj kot smo jih ugotovili z imunohistokemično metodo (51,15 ± 2,20 celice na mm^2), medtem ko je v postopku srebrenja reagiralo bistveno več celic, čeprav še vedno manj kot pri imunohistokemični reakciji (153,5 ± 0,32 celice na mm^2).

Ključne besede: veterinarska medicina; želodec; enteroendokrine celice; gastrin; somatostatin, serotonin; imunohistokemija; srebrenje po Grimeliusu; toluidinsko modrilo; prašič