

Cytomorphology Observations of Small Intestine Mucosa in Pig

Mirela E. CADAR

University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotechnologies, 3-5 Manastur Street, 400372 Cluj-Napoca, Romania; mirucadar@yahoo.com

Abstract. There were examined biological samples collected from anatomic segments of small intestine from 8 pigs one-year aged. Besides usual staining techniques (HE, Masson-Goldner trichromic) were effected specific staining for elastic fibers (Weigert) and for cells with acidophil granulations (Singh). Also, there were done impregnations for reticulin (Gomori) and for argentaffin enteroendocrine cells (Masson-Hamperl) and for argyrophile ones (Grimelius). Given the intestine histo-architecture common for mammals we find in pigs some particularities on epithelium level (presence of Paneth cells, abundance of enteroendocrine cells), on villi level (development of Brucke muscle) and on innervation level (development of Auerbach myenteric plexus in duodenum).

Keywords: small intestine, mucosa, pig, cytomorphology

INTRODUCTION

Concerning its microscopic structure the small intestine of domestic pig is, in many aspects, alike to this human one, but comparative physiology investigations marked out numerous particularities. These ones determined some researchers as Elias H. (1947), Titkemeyer C.W. and Calhoun M.L. (1955), Vodovar N. (1964) and many others to search the structural and ultra-structural substratum that could explain respective functional particularities of small intestine in pig and our study has this purpose.

MATERIALS AND METHODS

The biological samples have proceeded from 8 pigs one-year aged, slaughtered in Cluj private abattoir conditions. There were collected samples from many zones of duodenum, jejunum and ileum walls. The collected samples were fixed in 10% saline formaldehyde with *pH* 7. After inclusion in paraffin and 7 μm sectioning, there were effected next histological methods: staining with HE, staining with Masson-Goldner trichrome method, staining with Weigert resorcin-fuchsin for elastic fibers, Gomori silver impregnation for reticulin, Singh staining for cytoplasmic acidophil granulations, Masson-Hamperl argentaffin reaction (Singh variant) and Grimelius argyrophile reaction, last ones to mark out the enteroendocrine cells.

Uncolored samples, mounted in a non-fluorescent medium, were examined in UV light with 330 μm band to mark out the fluorescence induced by formaldehyde.

RESULTS AND DISCUSSION

The hito-architecture of small intestine wall in pig comprises the same four coats: mucosa, submucosa, muscularis and serosa) composed of same tissues as in majority of mammals. Thereby, we shall point out only these structures' details, which can be considered as particular for this species.

On duodenum level we find mucosa average thickness of 960 μm , comparatively to pigs aged of 200 days in which Vodovar N. (1964) noticed values of 835-1195 μm .

At this level, the intestinal villi were especial polymorph, predominating those leaf-like ones, their average height being 550 μm .

Some authors have contested the existence of circular folds (plicae circulares, Kerkring valves). We have observed that these circular folds are absent only in proximal portion of duodenum and were present in its distal half, fact mentioned also by Banks W.J. (1986), Cornilă N. and Manolescu N. (1995) and Samuelson D.A. (2007). At these folds' formation participate all mucosa layers, and in their ax penetrates the submucosa connective-vascular-nervous tissue. As particular aspect we observed that on this level the mucosa muscularis was thicker than in duodenum wall among folds.

The duodenal epithelium crypts among villi, which form Lieberkühn intestinal glands, comparatively to carnivorous ones, are more rare with sinuous route and predominantly oblique orientation. The average thickness of glandular layer was 380 μm .

In duodenal mucosa epithelium were present in variable proportions many cellular types: absorptive cells, goblet cells, regenerative cells, enteroendocrine cells and Paneth cells.

The absorptive cells present variable sizes depending on occupied position. Thus, in the Lieberkühn glands' epithelium they are small, cubical, have 25 μm average height at villi basis and reach 35 μm maximal height on the villi apex. This aspect is in relation with their vital cycle process during about 5 days, fact emphasized by Trier J.S. (1963) using radioactive isotopes' marking. The striated plateau of absorptive cells is very thin in those ones from villi basis (under 0.5 μm) and reaches 1.5 μm in those ones from villi apex. On the apical pole of absorptive cells in pigs, studies in electronic microscope marked out about 5,000 microvilli, 1-1.14 μm long and 20-50 nm thick (Palay S.L., Karlin L.J., 1959).

The goblet cells are relatively rare toward villi apex becoming more numerous in the profound region of Lieberkühn glands. Their vital cycle is appreciated to be about 3-4 days (Trier J.S., 1963).

The regenerative cells appear small, polyhedric, with ovoid euchromatic nucleus and are situated on basal membrane in intercellular spaces among absorptive and goblet cells, with priority in inferior half of Lieberkühn glands. In this zone can be observed numerous mitotic figures. Functionally, these cells are intestinal stem cells (Samuelson D.A., 2007).

Enteroendocrine cells with argentaffin granulations are present especially in the profound half of Lieberkühn glands, where in a conventional field covering an area of 600 μm^2 , we have observed in average 10 of these cells. At villi basis there were only 2-3 cells, and toward villi apex these cells were very rare. A similar repartition has presented also the enteroendocrine cells with argyrophile granulations.

Practicing successively two impregnation methods on the same sections we observed, as also has communicated Singh I. (1968) in human, dog, rabbit and guinea pig duodenum, the presence of three enteroendocrine cell types: with argentaffin granulations, with argyrophile granulations and a third one with both granulation types. The impregnation methods do not permit a certain identification of enteroendocrine cell types depending on their secretion and functions, and we can consider only that cells with argentaffin granulations are of EC₁ cell type (with serotonin and P-substance secretion), as well the EC₂ cell type (with serotonin and motilin secretion)(Lundqvist M. *et al.*, 1990).

Of cells' group with argyrophile granulations are part a great number of cellular types, which can be individualized only by immunocytochemistry methods, as are IG cells (gastrin), S cells (secretin), I cells (cholecystokinin), K cells (gastric inhibitory polypeptide), N cells (neurotensin) etc. (Olinici C.D., Călușer I., 1988).

Recent researches demonstrated that while argentaffin reaction is given by some aldehydes and biogen amines (for example serotonin), the argyrophile reaction is given by the A-chromogranin presence (Bankroft J.D., Stevens A., 1996).

On uncolored sections examined in UV light appeared with a yellow fluorescence the enteroendocrine cells, alike as number and disposition with those ones emphasized by argentaffin reaction. The researches effected by Falk B. *et al.* (1962) have specified that is about fluorescence induced through fixation with formaldehyde, the 5-hydroxitriptamin (serotonin) being converted in 3,4-dihydroxi-beta-carbolin, a derivative responsible of mentioned fluorescence. As concerns the Paneth cells, numerous authors admitted that these ones are found only in Lieberkühn glands from duodenum only in human, primates, equidae, rodents, are missing in leporidae, suinae and carnivorous species (Adlersberg L., 1955; Trier J.S., 1963; Banks W.J., 1986; Giraudet P. *et al.*, 1977; Samuelson D.A., 2007).

As contrary, Sloss M.W. (1954), cited by Vodovar N., has signalized the Paneth cell presence in pig, and Laumonier R. and Metayer J. (1967) in a study concerning exclusively these cells in normal and pathological status have affirmed their presence in majority of mammals, in fishes, reptiles and their absence or very reduced number in carnivorous species.

Using staining method proposed by Singh for the cells with acidophilic granulations (Mureşan E., Gaboreanu M, Bogdan A.T., Baba A.I., 1974), we observed reduced number of Paneth cells (1-2 cells in profound extremity of some of Lieberkühn glands). Patzelt and Hallay (cited by Laumonier R. and Metayer J., 1967) have observed that species that present great number of enteroendocrine cells have few Paneth cells.

The connective tissue of lamina propria from villi ax and among Lieberkühn glands is rich in cells especially peri-glandular. Particular for suina species is that among fibroblasts, lymphocytes, plasmocytes and macrophages are predominant the eosinophilic granulocytes. These ones form a dense peri-glandular and mucosa muscularis network, and in villi just under basal membrane. From here start transversal fibers, which end on central chyloferous vessel. Elastic fibers are abundant peri-glandular and in mucosa muscularis.

The central chyloferous vessel is more sizable in pig than in other species, is unique in conic villi and multiple in those leaf-like or soldered ones.

Different of solipeds and ruminants, in which capillaries of villi arteriole emerge from its basis, in domestic pig capillaries emerge from villi apex.

Another particularity of intestinal villi in domestic pig is the important development of Brucke muscle, its smooth fibers arriving until villi apex.

The lymphoid formations from lamina propria are numerically reduced, shaped in rare lymphoid follicles with small sizes.

Mucosa muscularis has structure and disposition common with those of other mammals, its average thickness being 25 µm and reaches 40-50 µm in circular folds.

Submucosa presented an average thickness of 500 µm. The fibrous connective tissue is reduced, forms thin walls in which is situated the submucosa blood and lymphatic vascular plexus as well small vegetative nervous ganglions from Meissner plexus. In rest, this coat is occupied by mucous tubular acini of Brunner glands. Particular for this species is that beside mucous acini are also rare serous acini. Among acinous cells were found also enteroendocrine cells numerically reduced.

Muscularis coat had a uniform thickness on all duodenum length, the inner circular layer (200 µm) being twice thicker than those outer longitudinal one (125 µm). Particular aspect was the presence of a myenteric vegetative nervous plexus (Auerbach plexus) very developed, being an almost continuous layer of neurons and nervous fibers between the two muscular plans. In this species, this structural aspect can be correlated with unusual fast

transit of aliments through duodenum toward jejunum, during 4-6 sec (Cotruț M., 1975). In the same way, we can also interpreted the abundance in duodenum of enteroendocrine cells, some of their secretion products being stimulators of intestinal peristalsis as serotonin, motilin, P-substance, VIP (vasoactive intestinal peptide).

The peritoneal serosa has 80 μm thickness, from which over 70 μm represent lamina propria of mesothelium and subserosa loose connective layer with its vascular-nervous plexus.

For the jejunum-ileum segment we shall mention only the structural differences given to duodenum one. Thus, Kerkring valves were completely circular, taller and more numerous in the first half of jejunum, then in ileum were rare and in its distal segment were missing. Intestinal villi were likewise polymorph, but taller and denser, Vodovar N. (1964) estimating their density at 1000-1200/cm². Lieberkühn glands were denser and longer in jejunum, and in ileum the glandular layer became discontinuous because of numerous lymphoid formations from inter-glandular lamina propria. Both in covering epithelium and in that one of Lieberkühn glands the goblet cells became more numerous as we bring closer of ileocecal valve. Paneth cells were missing, but those enteroendocrine ones were so numerous as in duodenum, becoming more rare in ileum.

The mucosa muscularis was interrupted here and there by the lymphoid formations, which extended from submucosa in inter-glandular lamina propria of mucosa. Macroscopically, in the small intestine of domestic pig can be observed about 30 Peyer patches elongated, big, especially in caudal half of jejunum. Microscopically, in front of them, the villi were rare or absent, and in epithelium can be observed the presence of some flattened cells, which are antigen presenting cells, named M cells (microfold cells).

The lymphoid formations' abundance determines that jejunum-ileum submucosa thickness to be almost double than in duodenum. The muscularis became thicker toward caudal extremity of small intestine and the inner and outer layer presented equal thickness. The serosa does not present differences given to that one of duodenum.

CONCLUSIONS

The histo-architecture of small intestine wall in domestic pig presents structural plan common to mammals, with some particularities:

- intestinal villi are very polymorph, predominantly leaf-like, have Brucke muscle very developed and a different capilarization of villi arteriole;
- enteroendocrine cells are particular numerous, especially in duodenum, both those ones with argentaffin granulations and those ones with argyrophile granulations;
- Paneth cells are present in reduced number only in Lieberkühn glands of duodenum;
- Auerbach myenteric nervous plexus is very developed especially in duodenum.

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