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Research Article

Serotonin production of the developing gastrointestinal tract of human embryos in 6th gestation week

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Abstract

Background: Local regulation of gastrointestinal tract digestion is performed by a large number of hormones produced by the mucosal enteroendocrine cells. Some of the earliest differentiating cells in the gastrointestinal tract are enteroendocrine cells. Serotonin-producing cells - EC cells are found mostly in the stomach and duodenum.

Aim: The aim of our study is to establish the presence and to make morphological and morphofunctional characteristic of EC cells in the developing gastrointestinal tract of a human.

Materials and methods: Our study was performed with biopsy specimen from human stomach and duodenum and fragments of gastrointestinal tract of human embryos 6th gestation week, studied by immunohistochemical, electron microscopy and morphometric methods.

Results: EC cells have already been differentiated in the 6th gestation week. Embryonic EC cells had identical characteristics with those of adults. They were in two morphofunctional conditions: stage of increased synthesis and stage of relative secretory rest.

Conclusion: In the early embryonic period - 6th gestation week EC cells have already been differentiated. The occurrence of EC cells with hormonal production prior to the definitive differentiation of tissues presupposes participation of serotonin in the digestive tube histogenetic processes.

Keywords

serotonin, 5-HT, EC cells, gastrointestinal tract, human embryo

Introduction

Local regulation of digestion is performed by a large number of hormones produced by the enteroendocrine cells in its mucosa. Some of the earliest described cells are the serotonin-producing cells (Zavidcic et al. 1976; Simonsson et al. 1988). Some of the earliest differentiating cells in the wall of the future gastrointestinal tract are enteroendocrine cells. In birds they have been identified on day 9 of incubation (Rawdon et al. 1999). In rats they are found on 18th day gestation (Ono et al. 1994), and in humans - in 8th gestation week (Stein et al. 1983). Studying the duodenum of human embryos Matsumoto et al. (2002) also established the presence of enteroendocrine cells in 8th gestation week. The presence of secretory granules suggests the participation of endocrine cell differentiation processes in the duodenal wall.

Serotonin (5-hydroxytryptamine, 5-HT) is a substance with various functions in the human body. It is involved in the regulation of a number of physiological processes, it also affects mental function. Serotonin (5-hydroxytriptamin, 5-HT) was found by V. Erspaner et al. in 1930. In 1951 serotonin was synthesized chemically (Yu et al. 2001). The total amount of serotonin in the human body is about 10 μg. 95% of it is in the gastrointestinal tract, and the remaining 5% in platelets and the central nervous system. Serotonin is an indole derivative, which belongs to the group of biogenic amines. It is formed by essential amino acid L-tryptophan. The enzyme tryptophan-5hydroxylase, which has two isoenzymes, performs a key role in serotonin synthesis. One of the enzymes is contained in the neurons and the other in the serotonin-producing EC cells in the gastrointestinal tract (Walther et al. 2003; Gershon et al. 2007; Li et al. 2011; Cho et al. 2014). In the CNS, a large number of neurons located in rafalnite nuclei, reticular formation, limbic system contain serotonergic neurons. Serotonin affects the regulation of body temperature, blood pressure, is related to higher nervous functions such as mood, sleep, memory, cognitive processes, food and sexual behaviors. Sunlight triggers its synthesis in the pineal gland and thus serotonin is involved in the regulation of biological rhythms in the body.

As a gastrointestinal hormone serotonin participates in the regulation of motility, secretion of the glands, sensitivity to pain (Voutilainen et al. 2002; Niesler et al. 2003; Hansen et al. 2008). Serotonin-producing cells - EC cells are found throughout the entire gastrointestinal tract but mostly in its proximal regions – stomach, duodenum, jejunum (Bruta et al. 2021). In the stomach they are located mainly in the antral and rarely in the corpus mucosa (Yacoub et al. 1996; Takahashi et al. 1998). Serotonin has been linked to cellular proliferation in several types of tissues, including vascular smooth muscle, neurons, and hepatocytes. Activation of serotonin receptors on distinct cell types has been shown to induce intracellular proliferation pathways. In the gastrointestinal tract, potentiation of serotonin signaling results in enhanced intestinal epithelial proliferation, and decreased injury from intestinal inflammation. Furthermore, activation of the type 4 serotonin receptor on enteric neurons leads to neurogenesis and neuroprotection in the setting of intestinal injury (Shah et al. 2021).

The aim of the present study is to establish the presence and to make a morphological and a morphofunctional characteristic of serotonin-producing EC cells in the human developing gastrointestinal tract.

Materials and methods

Our study was performed with biopsy specimen from the mucosa of human stomach and duodenum and small pieces of embryonic gastrointestinal tract of human embryos in 6th gestation week. The experimental material was studied by immunohistochemical, transmission electron microscopy and morphometric methods.

Biopsy specimen from adult stomach: corpus et antrum gastricum; and from duodenum, pars superior duodeni was obtained through fibrogastroscopic study of 15 female patients aged 45–72 years from the Clinic of Gastroenterology at 'St.George' university hospital and 'St. Pantheleymon' General hospital in the town of Plovdiv. These patients lacked endoscopic data for pathological changes in the gastric mucosa.

Material from 16 human embryos in 6th gestation week was prepared through vacuum extraction in artificial discontinuation of regular pregnancy at the Clinic of Obstetrics and Gynecology at 'St.George' university hospital. Determination of gestational week was performed not by anamnestic data from the patients but by morphological characteristics of the fragments from the aborted fetus extremities.

Immunohistochemical study

Biopsy specimen from adult gastrointestinal tract and fragments of 6th gestation week human embryo gastrointestinal tract was studied by immunohistochemical reactions for serotonin. The immunohistochemical analysis **(IHC)** was performed by the ABC method through rabbit ABC Staining System (Santa Cruz Biotechnology, USA) with the corresponding primary antibody.

Biopsy specimen and embryonic material for the IHC study we fixated in Buen solution for 24 hours and it included paraffin. Immunohistochemical reactions were performed according to the ABC method through rabbit ABC Staining System (Santa Cruz Biotechnology, USA) with the respective primary antibody. The material was fixed in a Buen solution for 24 hours and contained paraffin. The primary serotonin antibody (rabbit polyclonal antibody, MAB352 srotonin - Chemicon USA) was diluted in PBS in 1:200 ratio. We used a semi-quantitative evaluation method for the obtained results. The specificity of IHC reactions for each studied antigen was confirmed by negative controls in which the specific antibodies were substituted for a buffer (PBS) or normal non-immune

serum. Observation and photo documentation of microscopic preparations was performed with digital photo microscopic camera of a light microscope "Olympus BX51".

TEM

The experimantal material for transmission electron microscopy was fixated in 4% glutaraldehyde, NaPO4 buffer 0,1M, with subsequent postfixation in 4% OsO4 and 0,2M S'collin buffer. It was dehydrated in ascending alcohol series and was included in durcopan. Observation and microphotography was performed on TEM 'Philips CM 12'.

Morphometric study

The ultrastructural images of twenty EC cells from adult and twenty EC cells from embryonic gastrointestinal tract was studied with morphometric methods for the calculation of two coefficients:

1. Saturation index of the secretory granules in the EC cells by the method of W. Creutzfeldt;
2. Cytoplasmic distribution index of the secretory granules by the method of S. G. Khomeriki and Morozov.

Using morphometric image analysis software ImageJ 1.8.0 we counted the number of the secretory dranules in serotonin-producing EC cells (ImageJ for Windows 2021).

Saturation Index (SI) of the secretory granules in the EC cells was determined by the method of W. Creutzfeldt. The granules were determined as 1 - empty; 2 – half-empty (with small quantity of material); 3 –half-full and 4 – full. The saturation index of the granules of each EC cell was determined by multiplying the number of granules at each level of saturation by the corresponding coefficient (1, 2, 3, 4) and dividing the obtained value by the sum of the granules in the cell (Creutzfeldt et al. 1976).

Cytoplasmic Distribution Index (CDI) of the secretory granules was calculated by the method by Khomeriki and Morozov. The microphotographs of the different EC cells performed with the same magnification were divided into control areas of 0,5–0,25 cm^2 each. The number of the areas with high granule concentration (2 and more granules) – A2 and the number of the areas with low granule concentration (1 or no granules) – A1 were determined. The ratio between A2 and A1 represents the coefficient of granule distribution (Khomeriki et al. 1986).

The statistical analysis of the results was carried out in accordance with the conventional standard statistical procedures using computer statistical analysis by SPSS, version 26.0 for Microsoft Windows XP.

Data availability

Results gastrointestinal tract of adults

IHC reaction to serotonin found serotonin-positive cells in the biopsy material. Some of these are regularly scattered among the cells of the covering epithelium. Others are located in the neck and body of the corpus and pyloric glands. Serotonin-positive cells have a conical shape. Some of them do not reach the lumen of the gland. These are "closed" type cells. Other cells are extended, and their apical part reaches the lumen of the glands. These are "open" type cells of endocrine and, probably, paracrine action. Expression of serotonin is located in the enlarged basal part of cells (Fig. 1).

TEM

The secretory granules of the EC cells are polymorphic with rod-like or biconcave shape and a narrow light halo. Their electron density is high. We detected the presence of two types of EC cells – EC1 and EC2 type. EC1 cells were located mainly in the stomach. Their granules were polymorphic, mostly prolonged or oval. Their size was 200–300 nm. They contained serotonin and substance P (Fig. 2). EC2 cells were located in the duodenum. There were mainly oval shaped granules sized 200–400 nm in their cytoplasm. EC2 cells contained serotonin and motilin (Fig. 3).

Morphometric study

Based on their ultrastructucal characteristic, and the coefficients identified by morphometric researches, we found that EC cells revealed different morphofunctional stages.

In the stage of increased synthesis, EC1 cells had big loose young granules that were detatched from the trans-surface of the dictiosomes. The young granules of the EC2 cells were numerous and filled a substance with low electron density. They increased their sizes and formed a gritted progranule with osmiffil covering membrane. During the next ripening, the core of the granules became homogeneous, and the electron density was high. The saturation index of the granules in adults (SIA) was 3.23±0.3. The changes in the distribution index in the granules in the cytoplasm showed an activation of the secretory processes. In the stage of increased synthesis, the granules were uniformly dispersed in the whole cytoplasm. The number of the A1 fields with low density of granules was lower than the number of A2 fields with high density of the granules. The cytoplasmic distribution index of the granules in adults (CDIA) was 0.70±0.3 (Fig. 4).

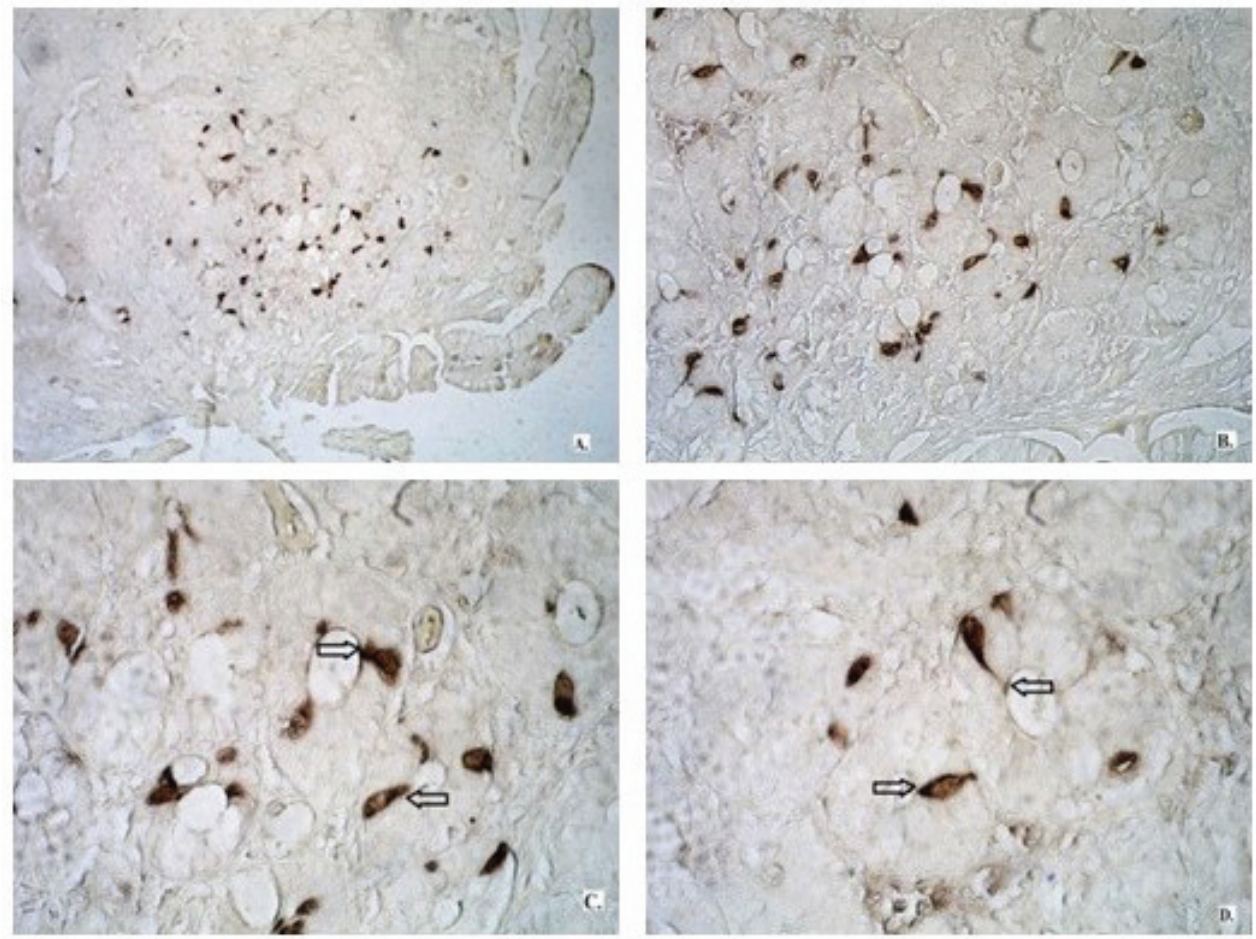

Figure 1. Gastrointestinal tract of adults. IHC. Paraffin preparation. **A.** Serotonin-positive cells in biopsy specimen from gastric corpus. Magn × 10; **B.** Serotonin-positive cells in the area of the fundus of glands. Magn. × 20; **C, D.** Serotonin-positive cells from 'closed' type and from 'open' type. Magn × 40.

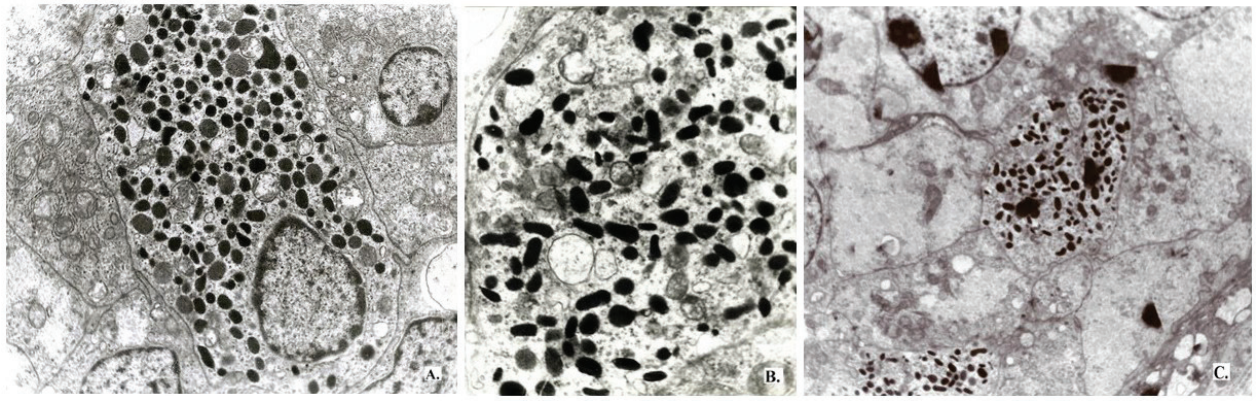

Figure 2. Gastrointestinal tract of adults. TEM. Biopsy specimen from gastric corpus mucosa. **A.** Serotonin-producing EC1 cell. Micr. Magn. x 2350; Photogr. Magn. x 11750; **B.** Fragment of serotonin-producing EC1 cell. Micr. Magn. x 5000; Photogr. Magn. x 25000; **C.** Fragments of two serotonin-producing EC1 cells. Micr. Magn. x 1500; Photogr. Magn. x 7500.

In the stage of relative secretory rest, the cytoplasm of the EC cells was filled with ripe granules that were full of secretion. The saturation index of the granules in adults (SIA) was 3.45±0.3. In the stage of relative secretory rest, the granules occupied mainly the basal part of the cell. The number of the A1 fields with low density of the granules increased and the cytoplasmic distribution index of the granules in adults (CDIA) started to decrease – 0.38±0.3 (Fig. 5).

In the stage of increased secretion, the disintegration of the granules began. Their diameter increased, the electron density of the core decreased, the halo of the membrane disappeared. Remnants of a dispersed substance could be found later in the core of the granule. In the end there was only a membrane skeleton left. The saturation index of the granules in adults (SIA) was 2.95±0.2. In the stage of increased secretion, the granules were in their highest

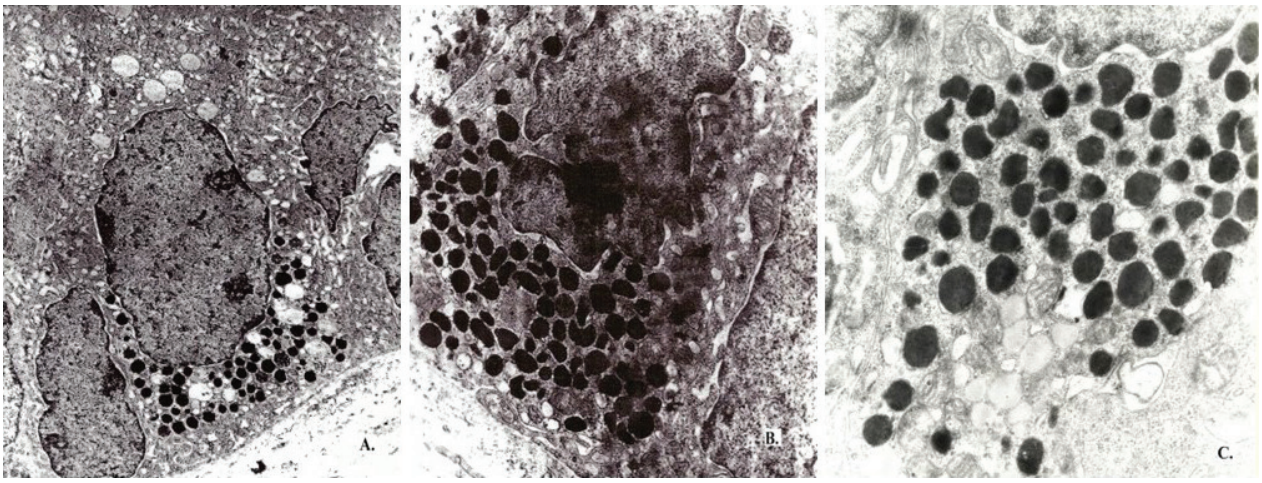

Figure 3. Gastrointestinal tract of adults. TEM. Biopsy specimen from duodenal mucosa. **A.** Serotonin-producing EC2 cell. Micr. Magn. x 1500; Photogr. Magn. x 7500; **B.** Serotoninproducing EC2 cell. Micr. Magn. x 2350; Photogr. Magn. x 11750; **C.** Fragment of serotoninproducing EC2 cell. Micr. Magn. x 5000; Photogr. Magn. x 25000.

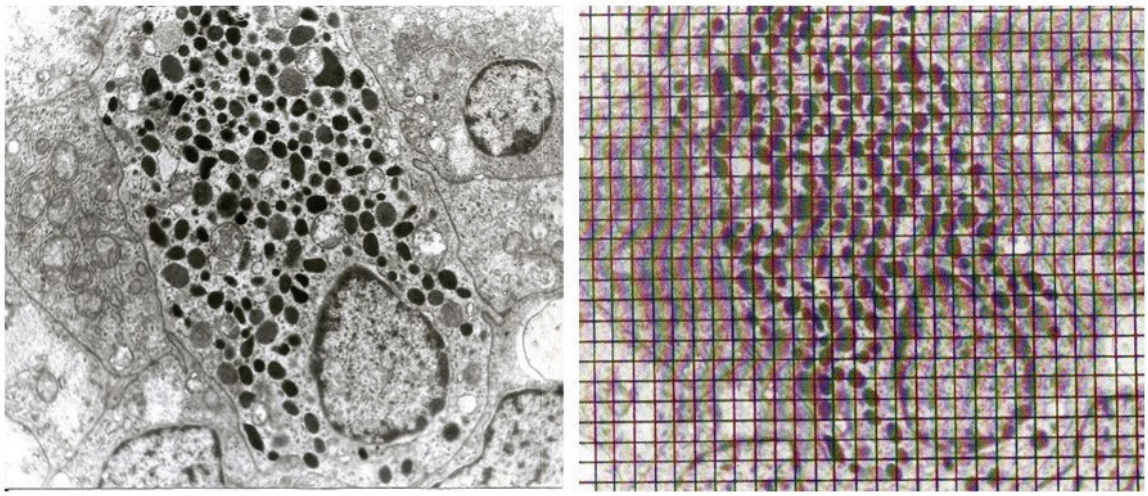

Figure 4. Gastrointestinal tract of adults. Serotonin-producing EC cell in the stage of increased synthesis. Micr. Magn. x 2350; Photogr. Magn. x 11750.

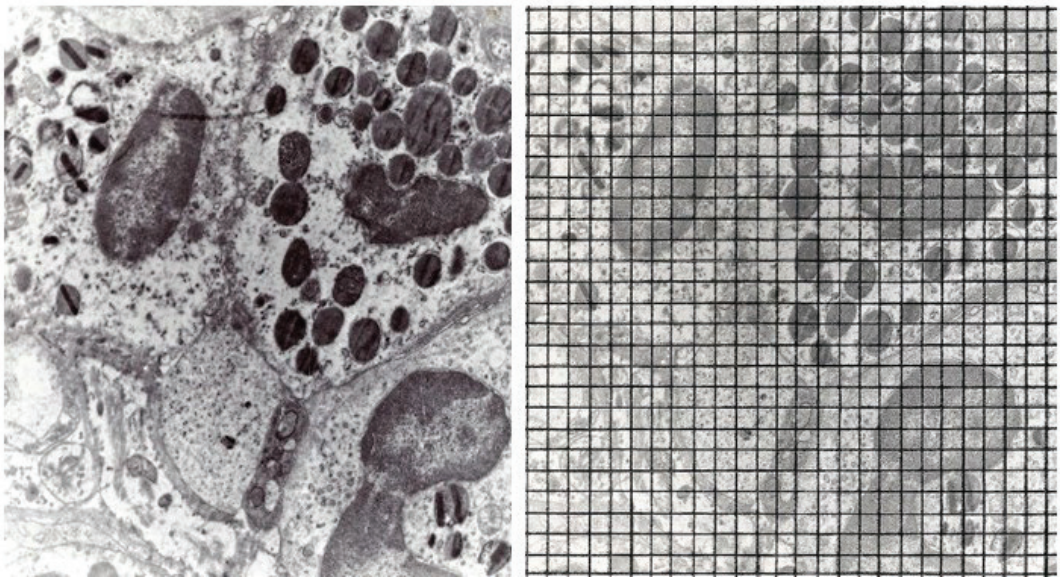

Figure 5. Gastrointestinal tract of adults. Serotonin-producing EC cell in the stage of relative secretory rest. Micr. Magn. x 2350; Photogr. Magn. x 11750.

concentration. There were A1 mostly fields with low electronic density. The cytoplasmic distribution index of the granules in adults (CDIA) decreased to 0.36±0.2 (Fig. 6).

Gastrointestinal tract of human embryo 6th gestational week

IHC reaction for serotonin was positive in the small intestine of human embryos in 6th gestation week. The reaction was positive in a small number of cells. These were scattered singularly between the resorptive cells in the epithelial lining of the small intestine villi. Serotonin expression was localized in the basal part of the cells (Fig. 7).

TEM

EC cells in the gastrointestinal tract during the early embryogenesis in humans - 6th gestation week, showed high degree of differentiation. Their morphological characteristics were not very different from the characteristics of the EC cells in adults. The nuclei of the embryonic EC cells had well-outlined nuclear envelope and finely dispersed chromatin. Secretory granules occupy mainly the basal portion of the cell. And this leads to ultrastuctural polarization of embryonic EC cells. The mitochondria were small with increased matrix density. The rough endoplasmic reticulum was diffusely scattered.

The type of EC cells we observed in the embryonic stomach and small intestine was the same as in adults. We detected the presence of EC1 - serotonin and substance P producing cells (Fig. 8); and EC2 cells - serotonin and motilin producing cells (Fig. 9).

Morphometric study

Morphometric analysis of the embryonic EC cells found cells only in two morphofunctional stages: stage of increased synthesis and stage of relative secretory rest.

EC cell in stage of increased synthesis At the stage of increased synthesis, half-empty granules with scarce granular material predominated in EC cell cytoplasm. Their contents had decreased electron density. The saturation index of the granules in embryos (SIE) was 3,20±0.3. At the stage of increased synthesis, granules were evenly dispersed throughout the cytoplasm. The number of fields with the low density of the granules A1 was smaller than the number of fields with high density A2. The cytoplasmic distribution index of the granules in embryos (CDIE) was 0.72±0.3 (Fig. 10).

EC cell in stage of relative secretory rest At the stage of relative secretory rest, the cytoplasm of EC cells was filled with mature granules with maximum accumulated secretion. The saturation index of the granules in embryos (SIE) was 3.42±0.3. At the stage of relative secretory rest, granules occupied mainly the basal part of the cells. The number of fields with the low density of A1granules increased, and the coefficient of distribution of the granules had begun to decrease. The cytoplasmic distribution index of the granules in embryos (CDIE) was 0.40±0.3. (Fig. 11).

EC cells in the gastrointestinal tract of adults were situated in three morphofunctional stages: stage of increased synthesis; stage of relative secretory rest; stage of increased secretion.

EC cells in gastrointestinal tract of human embryos in the 6th gestation week were situated in two morphofunctional stages - in stage of increased synthesis and in stage of relative secretory rest.

A comparative morphometric analysis of embryonic EC cells with those of adults showed that embryonic cells had identical morphological characteristics with those of adults. There was no significant difference between SI and CDI of the two age groups – adults and embryos in stage of increased synthesis and in stage of relative secretory rest. (Table 1, Fig. 12).

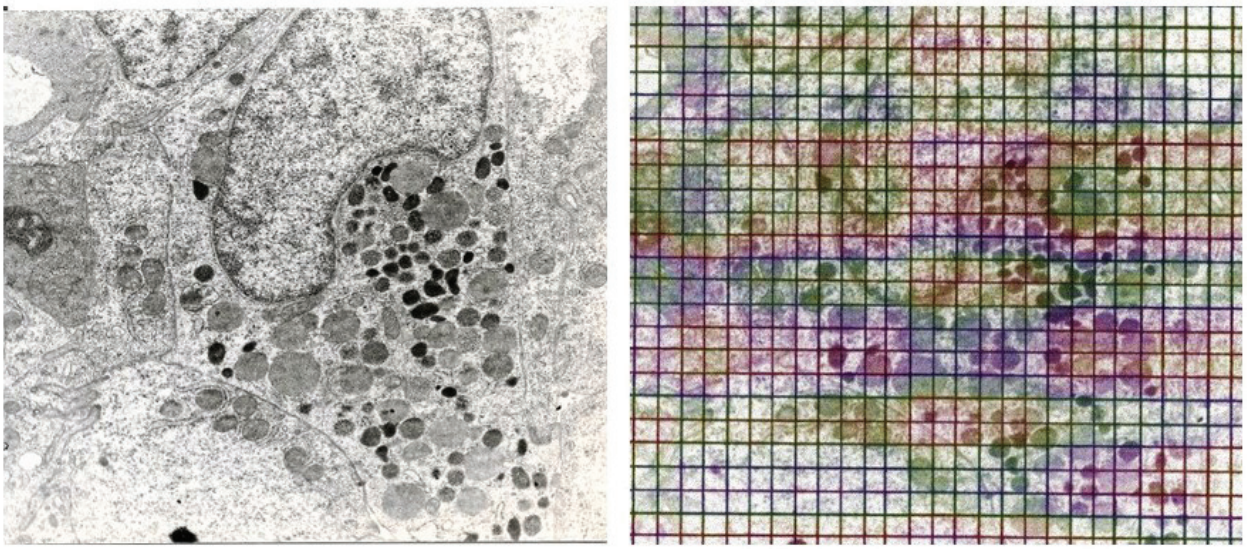

Figure 6. Gastrointestinal tract of adults. Serotonin-producing EC cell in the stage of increased secretion. Micr. Magn. x 2350; Photogr. Magn. x 11750.

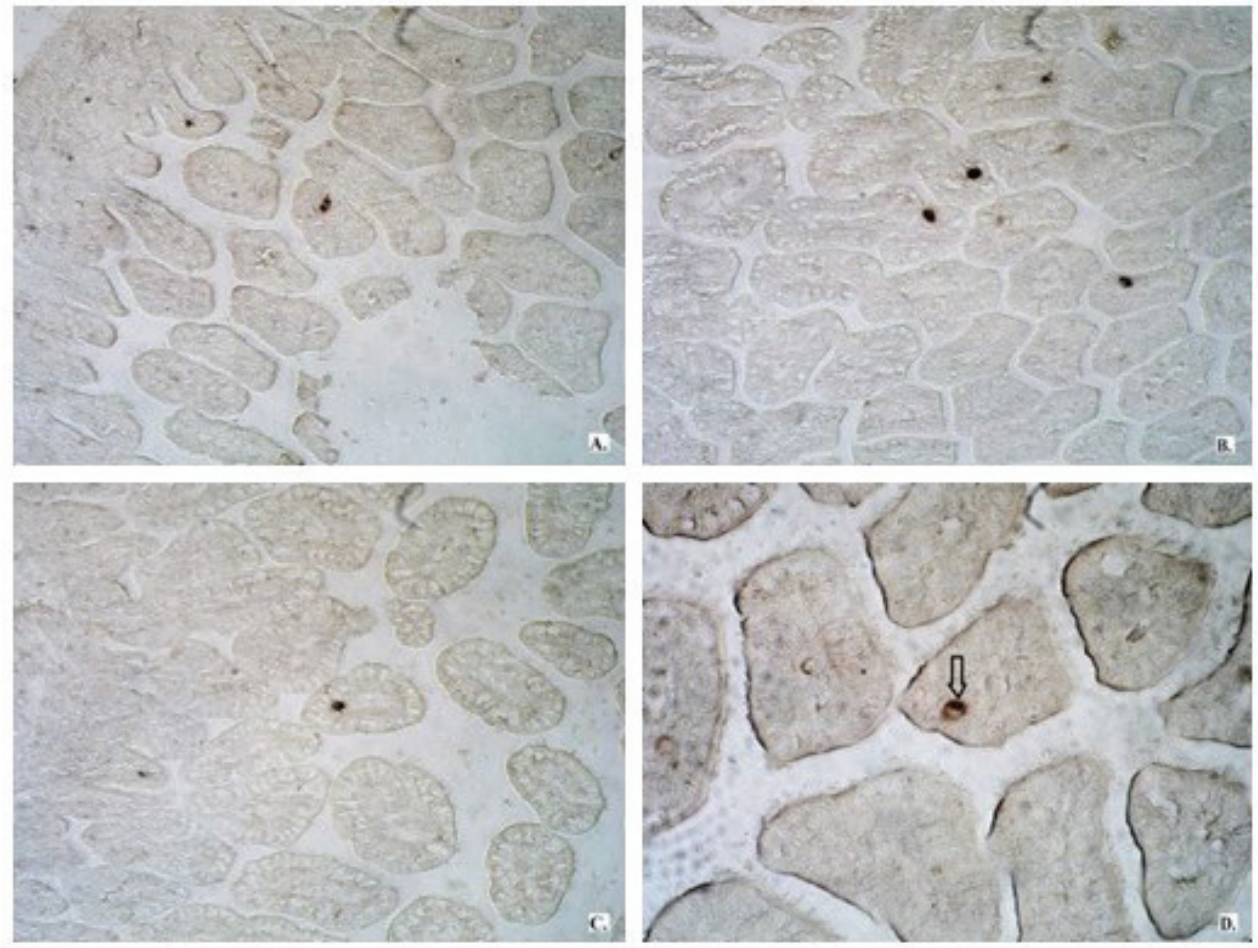

Figure 7. Small intestine of human embryo 6th gestational week. IHC. Paraffin preparation. Serotnin-positive cells in the covering epithelium of the small intestine villi and at the bottom of the crypts of Lieberkuhn. **A.** Magn. x 10; **B.** Magn. x 20; **C.** Magn. x 20; **D.** Serotonin expression in the basal part of the cells. Magn. x 40.

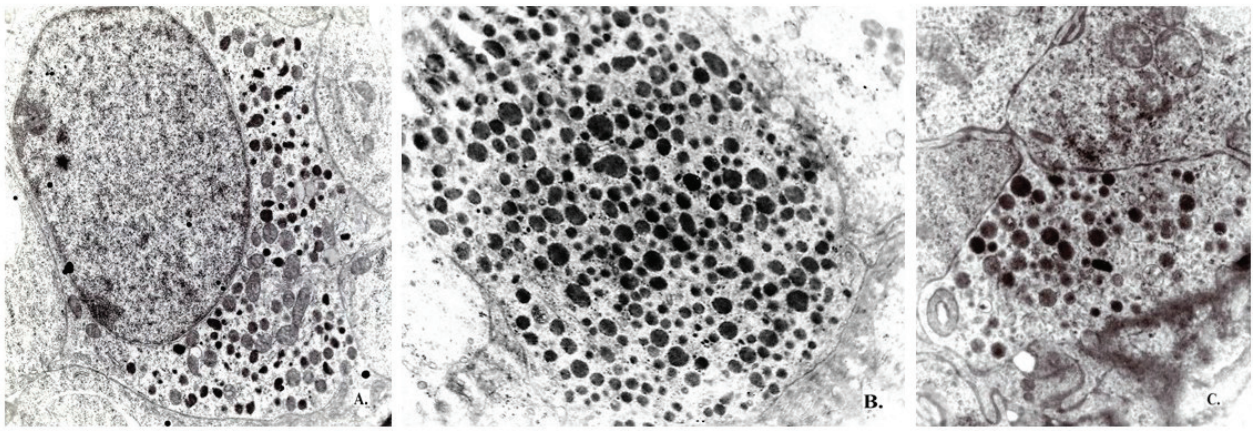

Figure 8. Gastrointestinal tract of human embryo 6th gestational week. TEM. **A.** Stomach**.** Serotonin-producing EC1 cell. Micr. Magn. x 2350; Photogr. Magn. x 11750; **B.** Small intestine. Fragment of serotonin-producing EC1 cell. Micr. Magn. x 3900; Photogr. Magn. x 19500; **C.** Stomach**.** Fragment of serotonin-producing EC1 cell. Micr. Magn. x 5000; Photogr. Magn. x 25000.

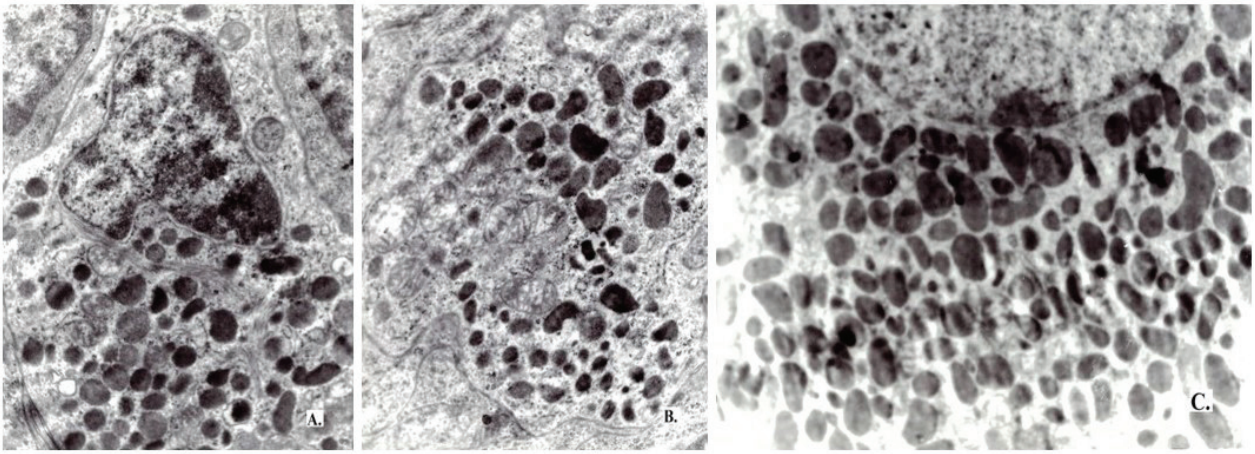

Figure 9. Gastrointestinal tract of human embryo 6th gestational week. TEM. **A.** Stomach**.** Serotonin-producing EC2 cell. Micr. Magn. x 5000; Photogr. Magn. x 25000; **B.** Small intestine. Fragment of serotonin-producing EC2 cell. Micr. Magn. x 5000; Photogr. Magn. x 25000; **C.** Stomach**.** Fragment of serotonin-producing EC2 cell. Micr. Magn. x 2950; Photogr. Magn. x 14750.

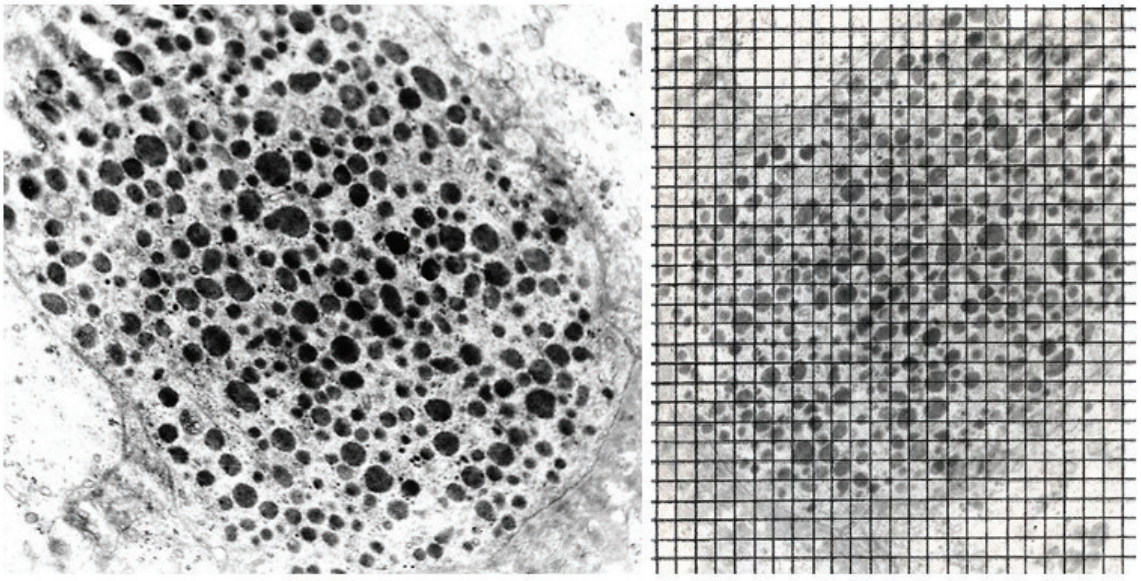

Figure 10. Gastrointestinal tract of human embryo 6th gestational week. Serotonin-producing EC cell in the stage of increased synthesis. Micr. Magn. x 5000; Photogr. Magn. x 25000.

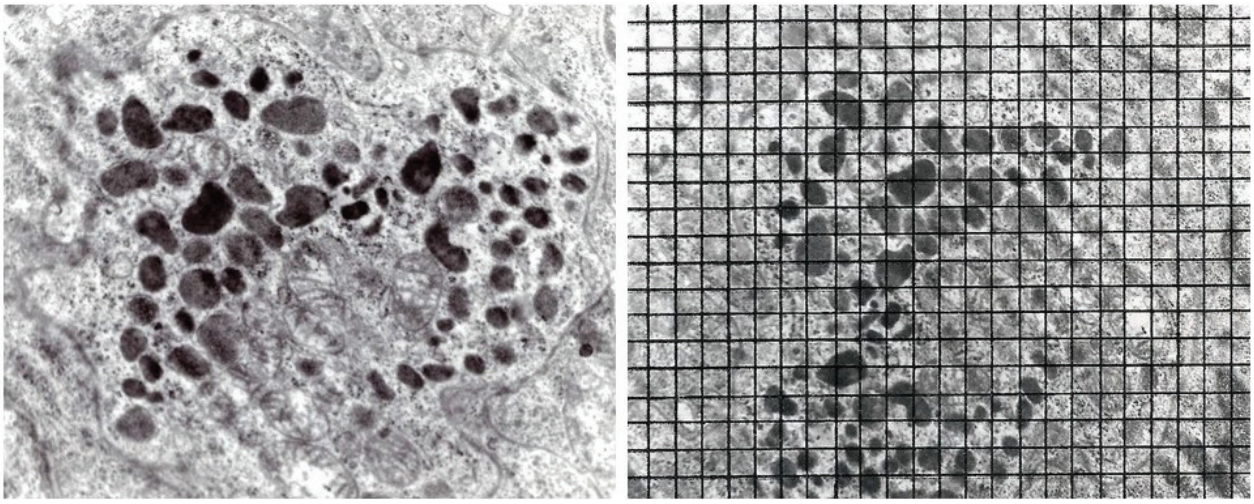

Figure 11. Gastrointestinal tract of human embryo 6th gestational week. Serotonin-producing EC cell in the stage of relative secretory rest. Micr. Magn. x 5000; Photogr. Magn. x 25000.

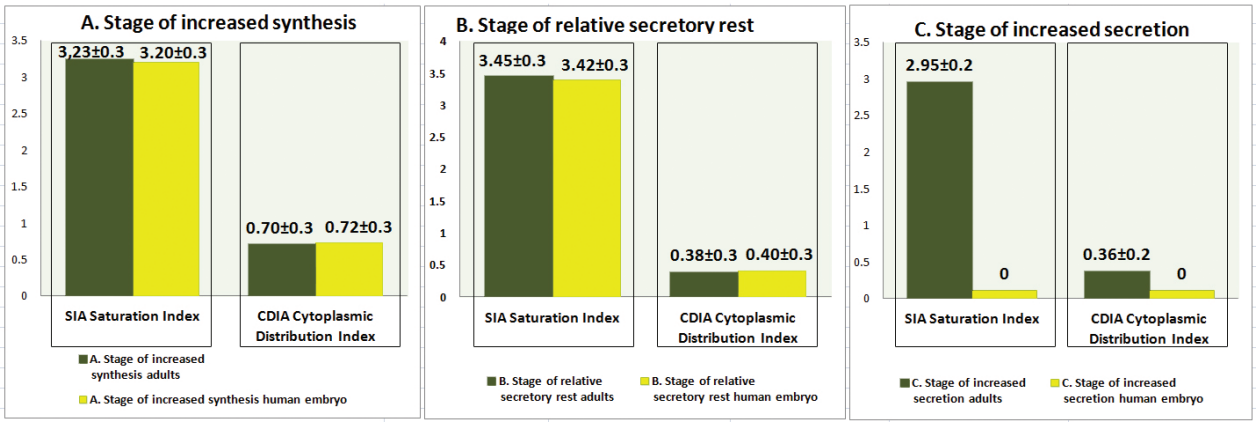

Figure 12. Graphical analysis of SI and CDI data of serotonin-producing EC cells from gastrointestinal tract of adults and human embryos 6th gestation week in the three morphofunctional stages.

Table 1. Comparative table of morphometric study of serotonin-producing EC cells from gastrointestinal tract of adults and human embryos in 6th gestation week.

Serotonin-producingn EC cells		Saturation index SI	Cytoplasmic distribution index CDI
Stage of increased synthesis	adults	3,23±0.3	0.70±0.3
	embryo	3.20±0.3	0.72±0.3
Stage of relative secretory rest	adults	3.45±0.3	0.38±0.3
	embryo	3.42±0.3	0.40±0.3
Stage of increased secretion	adults	2.95±0.2	0.36±0.2
	embryo	0	0

Discussion

In the biopsy material from gastric and duodenal mucosa we found a large number of EC cells scattered in the covering epithelium, in the fundic, pyloric glands and crypts of Lieberkuhn. By TEM we found the presence of serotonin-producing cells from two types: EC1 - serotonin and substance P producing cells; and EC2 cells - serotonin and motilin producing cells.

EC cells are positioned along the length of gastric glands, but especially in their basal compartments (Zavidcic et al. 1976; Simonsson et al. 1988). They have an oval or conical shape (Takahashi et al. 1998). The wide part of EC cells lies on the basal membrane of the glands or crypts of Lieberkuhn. EC cells are enteroendocrine cells in the gastrointestinal epithelium that release serotonin in response to a variety of stimuli including mechanical force. Mechanosensitive ion channel Piezo2 from EC cell's basal plasmalema is involved in EC cell mechanotransduction (Knutson et al. 2016). The narrow apical part of cells is directed towards the lumen of the gland (Rantala et al. 1996). EC cells have long processes which resemble neuronal processes with varicosities. Electron microscopic observations revealed rod-like, tortuous, oval, or round small pleomorphic granules in the long process bearing EC cells. The cell bodies and processes directly faced the crypt epithelial cells - including the enterocytes and goblet cells on one side and the basement membrane on the opposite side. The accumulation of the granules sometimes appeared within the cytoplasm on the side of the epithelial cells. These findings suggest that serotonin is released from the long processes of the EC cells and directly acts in a paracrine fashion on the crypt epithelial cells (Kuramoto et al. 2007).

The characteristics of secretory granules established by our study can be used as a database for comparison of pathological conditions in neoplastic processes. The normal EC cells have secretory granules with oval, elongation or biconcave shape and narrow halo. The secretory product is equally distributed and has a high electron density. Phenotypic characterization of EC cells in carcinoid tumors shows a deviation from normal morphology (Yu et al. 2001). Neoplastic EC cells have a large nucleus, a large number of mitochondria; a mixture of small vesicles, empty vacuoles and secretory granules with irregular electron density (Modlin et al. 2006; Kidd et al. 2008).

Our morphometric analysis of EC cells in adult showed different functional states: stage of increased synthesis, stage of relative secretory rest and stage of increased secretion. Ultrastructure morphometry allows to make detailed analysis of changes in different types of EC cells in diseases of the gastrointestinal tract and in the course of their therapy (D'Adda et al. 1996; Hirschowitz 1998; Bordi 2000; Bektaş 2012).

Morphogenetic processes in the formation of the gastrointestinal tract include the formation of primary gut, its elongation and sectoral differentiation of definitive organs: esophagus, stomach, small and large intestines.

The primitive gut tube is composed of two tissues: a luminal lining of endoblas-derived epithelium surrounded by an outer layer of splanchnic mesoderm. As development progresses, the splanchnic mesoderm undergoes smooth muscle differentiation, causing the gut tube to alter its gross morphology. Meanwhile, the uniform luminal epithelium undergoes regional specification along the anterior–posterior axis. For example, the lumen of the stomach is lined with a gastric columnar epithelium, which is required for secreting enzymes for the breakdown of food, while the lumen of the small intestine contains bulbous microvilli required for the absorption of nutrients (Theodosiou et al. 2003).

In our study the we found that in the 6th gestation week of embryonic development of the gastrointestinal tract differentiated EC cells are present.

They were located in the endoblast of the future stomach and small intestine. Ultastructural characteristic of embryonic EC cells from the EC1 and EC2 type was identical to those of serotonin-producing cells in adults. Comparative morphometric analysis indicated that embryonic EC cells were located in two morphofunctional stages: stage of increased synthesis and stage of relative secretory rest.

Oberg K et al. (1998) describes the gastric epithelium in the later period: 9th - 10th gestation week. Mitrović O et al. (2012) presented initial histological characteristics of the developing gasroinestinal tract in humans through the 10th gestation week.

Rindi et al. (2004) determined enteroendocrine cells of the small intestine as a highly specialized population of mucosal cells. Some of them – enterochromaffin EC cells packed serotonin in large granules with a dense core and in microvesicles similar to those synaptic (Rindi et al. 2004).

The endoblast of the small intestine begins to change after 5th gestation week. Between 5th and 8th gestation week the formation of the small intestine villi begins. Spence et al. (2011) described the onset of this process. It starts from the territory of the mesenchyme. Mesenchymal cells under the endoblast begin to group and to grow in the direction of the lumen. At first wide folds with a small height are formed. These folds gradually lengthen, become thinner and increase their number (Spence et al. 2011).

In our study IHC reaction for serotonin was positive for a small number of cells on the wall of the small intestine in 6th gestation week. Some of serotonin-positive cells were located singly between the resorptive cells in the covering epithelium of the small intestine villi, the other – at the bottom of shallow crypts of Lieberkuhn. They were found both along the villi as well as in their peak area. The small number of serotonin-producing cells was probably due to the unfinished processes of maturation in the small intestine wall. The localization of these cells, found by us, in the peak areas of the small intestine villi might be explained with the intensive processes of proliferation of stem cells in the developing crypts and epithelial cell migration along the radiant crypt-villus axis of the small intestine wall. The migration processes also take place in the mature small intestine. At the bottom of the intestine glands a small group of stem cells are located which provide several cellular phenotypes- resorptive, cup-like, endocrine. These cells constantly migrate to the adjacent villi (Pacha et al. 2000; Quinlan et al. 2006).

Stein et al. (1983) showed that enteroendocrine cells appear in the stomach, duodenum and pancreas of human embryos at the same time - the 8th gestation week. Through research with antichromogranin A antibody, a common marker for enteroendocrine cells, Mitrović O et al., (2012) found the occurrence of enteroendokrinni cells in 10th gestation week. In 10th gestation week somatostatin cells (D-type) appear; in 11th gestation week - serotonin (EC-type) and ghrelinproducin cells; and enteroendocrine cells secreting glucagon (A-type) appear in the 12th gestation week (Mitrović O et al. 2012).

Conclusion

Serotonin-producing EC cells in adult are in three morphofunctional stages: stage of increased synthesis; stage of relative secretory rest; stage of increased secretion. In early embryonic period - 6th gestation week week serotonin-producing cells have already been differentiated. The ultramicroscopic characteristic of the embryonic cells shows presence of EC1 and EC2 serotoninproducing enteroendocrine cells. Embryonic serotonin-producing EC cells are in two morphofunctional stages: stage of increased synthesis; stage of relative secretory rest. The occurrence of serotonin-producing enteroendocrine cells with signs of hormonal production prior to the definitive differentiation of tissues presupposes participation of the gastrointestinal hormones in the histogenetic processes in the digestive tube.

References

Bektaş M, Saraç N, Cetinkaya H, Törüner M, Erdemli E, Keskin O, Soykan I, Oktay EI, Korkut E, Ustün Y, Bahar K (2012) Effects of *Helicobacter pylori* infection and long-term proton pump inhibitor use on enterochromaffin-like cells. Annals of Gastroenterology 25(2): 123–127. [PMID: 24714139; PMCID: PMC3959398]

Bordi C, Azzoni C, Ferraro G, Corleto VD, Gibril F, Delle Fave G, Lubensky IA, Venzon DJ, Jensen RT (2000) Sampling strategies for analysis of enterochromaffin-like cell changes in Zollinger-Ellison syndrome. American Journal of Clinical Pathology 114(3): 419–425. https://doi.org/10.1093/ajcp/114.3.419

Bruta K, Vanshika, Bhasin K, Bhawana (2021) The role of serotonin and diet in the prevalence of irritable bowel syndrome: a systematic review. Translational Medicine Communications 6: 1. https://doi.org/10.1186/s41231-020-00081-y

Cho HJ, Callaghan B, Bron R, Bravo DM, Furness JB (2014) Identification of enteroendocrine cells that express TRPA1 channels in the mouse intestine. Cell and Tissue Research 356(1): 77–82. https://doi.org/10.1007/s00441-013-1780-x

Creutzfeldt W, Arnold R, Creutzfeldt C, Track NS (1976) Mucosal gastrin concentration, molecular forms of gastrin, number and ultrastructure of G-cells in patients with duodenal ulcer. Gut 17(10): 745–754. https://doi.org/10.1136/gut.17.10.745

D'Adda T, Annibale B, Delle Fave G, Bordi C (1996) Oxyntic endocrine cells of hypergastrinaemic patients. Differential response to antrectomy or octreotide. Gut 38(5): 668–674. https://doi.org/10.1136/gut.38.5.668

Gershon MD, Tack J (2007) The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 132: 397–414. https://doi.org/10.1053/j.gastro.2006.11.002

Hansen MB, Witte AB (2008) The role of serotonin in intestinal luminal sensing and secretion. Acta Physiologica 193(4): 311–323. https://doi.org/10.1111/j.1748-1716.2008.01870.x

Hirschowitz BI (1998) Clinical aspects of ECL-cell abnormalities. Yale Journal of Biology and Medicine 71(34): 303–310. https://doi.org/10.2165/00003088-199834040-00003

ImageJ for Windows 1.8.0. (2021) Joonas "Regalis" Rikkonen. https://www.airtel.co.ug/internetservice/airtelapp

Khomeriki SG, Morozov I (1986) Value of quantitative evaluation of the distribution of secretory granules in the cytoplasm of endocrine cells of the stomach. Archives d'anatomie, d'histologie et d'embryologie XC: 87–91. https://www.unboundmedicine.com/medline/citation/2871824/

Kidd M, Modlin IM, Gustafsson BI, Drozdov I, Hauso O, Pfragner R (2008) Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. American Journal of Physiology-Gastrointestinal and Liver Physiology 295(2): G260-G272. https://doi.org/10.1152/ajpgi.00056.2008

Knutson K, Wang F, Alcaino C, Bernard Ch, Farrugia G, Beyder A (2016) The mechanosensitive ion channel Piezo2 is selectively expressed in enterochromaffin (EC) cells of the human and mouse gastrointestinal mucosa. The FASEB Journal 30(1): 1254.5.

Kuramoto H, Kadowaki M, Sakamoto H, Yuasa K, Todo A, Shirai R (2007) Distinct morphology of serotonin-containing enterochromaffin (EC) cells in the rat distal colon. Archives of Histology and Cytology 70(4): 235–241. https://doi.org/10.1679/aohc.70.235

Li Z, Chalazonitis A, Huang YY, Mann JJ, Margolis KG, Yang QM, Kim DO, Côté F, Mallet J, Gershon MD (2011) Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. Journal of Neuroscience 31(24): 8998–9009. https://doi.org/10.1523/JNEUROSCI.6684-10.2011

Matsumoto A, Hashimoto K, Yoshioka T, Otani H (2002) Occlusion and subsequent recanalization in early duodenal development of human embryos: integrated organogenesis and histogenesis through a possible epithelial-mesenchymal interaction. Anatomy and Embryology (Berlin) 205(1): 53–65. https://doi.org/10.1007/s00429-001-0226-5

Mitrović O, Mićić M, Radenković G, Vignjević S, Đikić D, Budeč M, Breković T, Čokić V (2012) Endocrine cells in human fetal corpus of stomach: appearance, distribution, and density. Journal of Gastroenterology 47(11): 1212–1220. https://doi.org/10.1007/s00535-012-0597-9

Modlin IM, Kidd M, Pfragner R, Eick GN, Champaneria MC (2006) The functional characterization of normal and neoplastic human enterochromaffin cells. The Journal of Clinical Endocrinology & Metabolism 91(6): 2340–2348. https://doi.org/10.1210/jc.2006-0110

Niesler B, Frank B, Kapeller J, Rappold GA (2003) Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 310: 101–111. https://doi.org/10.1016/S0378-1119(03)00503-1

Oberg K (1998) Gastric neuroendocrine cells and secretory products. The Yale Journal of Biology and Medicine 71(34): 149–154. [PMID: 10461347; PMCID: PMC2578979]

Ono E, Doi Y, Furukawa H, Hirata K, Fujimoto S (1994) The differentiation of enteroendocrine cells of pre- and postnatal rats: light and electron microscopy and immunocytochemistry. Acta Anatomica (Basel) 149(2): 81–88. https://doi.org/10.1159/000147561

Pácha J (2000) Development of intestinal transport function in mammals. Physiological Reviews 80(4): 1633–1667. https://doi.org/10.1152/physrev.2000.80.4.1633

Quinlan JM, Yu WY, Hornsey MA, Tosh D, Slack JM (2006) In vitro culture of embryonic mouse intestinal epithelium: cell differentiation and introduction of reporter genes. BMC Developmental Biology 6: 24. https://doi.org/10.1186/1471-213X-6-24

Rantala I, Paronen I, Kainulainen H, Ala-Kaila K (1996) Enterochromaffin cell density in the gastric mucosa of patients with chronic renal failure. APMIS 104(5): 362–266. https://doi.org/10.1111/j.1699-0463.1996.tb00728.x

Rawdon BB, Andrew A (1999) Gut endocrine cells in birds: an overview, with particular reference to the chemistry of gut peptides and the distribution, ontogeny, embryonic origin and differentiation of the endocrine cells. Progress in Histochemistry and Cytochemistry 34(1): 3–82. https://doi.org/10.1016/S0079-6336(99)80004-7

Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E (2004) The "normal" endocrine cell of the gut: changing concepts and new evidences. Annals of the New York Academy of Sciences 1014: 1–12. https://doi.org/10.1196/annals.1294.001

Shah PA, Park CJ, Shaughnessy MP, Cowles RA (2021) Serotonin as a mitogen in the gastrointestinal tract: Revisiting a familiar molecule in a new role. Cellular and Molecular Gastroenterology and Hepatology 12(3): 1093–1104. https://doi.org/10.1016/j.jcmgh.2021.05.008

Simonsson M, Eriksson S, Håkanson R, Lind T, Lönroth H, Lundell L, O'Connor DT, Sundler F (1888) Endocrine cells in the human oxyntic mucosa. A histochemical study. Scandinavian Journal of Gastroenterology 23(9): 1089–1099. https://doi.org/10.3109/00365528809090174

Spence JR, Lauf R, Shroyer NF (2011) Vertebrate intestinal endoderm development. Developmental Dynamics 240(3): 501–520. https://doi.org/10.1002/dvdy.22540

Stein BA, Buchan AM, Morris J, Polak JM (1983) The ontogeny of regulatory peptidecontaining cells in the human fetal stomach: an immunocytochemical study. Journal of Histochemistry & Cytochemistry 31(9): 1117–1125. https://doi.org/10.1177/31.9.6136542

Takahashi Y, Imanaka T, Takano T (1998) Spatial pattern of smooth muscle differentiation is specified by the epithelium in the stomach of mouse embryo. Developmental Dynamics 212(3): 448–460. https://doi.org/10.1002/(SICI)1097-0177(199807)212:3%3C448::AID-AJA12%3E3.0.CO;2-X

Theodosiou NA, Tabin CJ (2003) Wnt signaling during development of the gastrointestinal tract. Developmental Biology 259(2): 258–271. https://doi.org/10.1016/S0012-1606(03)00185-4

Voutilainen M, Juhola M, Pitkänen R, Färkkilä M, Sipponen P (2002) Immunohistochemical study of neuroendocrine cells at the gastric cardia mucosa. Journal of Clinical Pathology 55(10): 767–769. https://doi.org/10.1136/jcp.55.10.767

Walther DJ, Bader M (2003) A unique central tryptophan hydroxylase isoform. Biochemical Pharmacology 66(9): 1673–1680. https://doi.org/10.1016/S0006-2952(03)00556-2

Yacoub WR, Thomson AB, Hooper P, Jewell LD (1996) Immunocytochemical and morphometric studies of gastrin-, somatostatin- and serotonin-producing cells in the stomach and duodenum of patients with acid peptic disorders. Canadian Journal of Gastroenterology and Hepatology 10(6): 395–400. https://doi.org/10.1155/1996/245908

Yu PL, Fujimura M, Hayashi N, Nakamura T, Fujimiya M (2001) Mechanisms in regulating the release of serotonin from the perfused rat stomach. American Journal of Physiology-Gastrointestinal and Liver Physiology 280(6): G1099–G1105. https://doi.org/10.1152/ajpgi.2001.280.6.G1099

Zaviacíc M, Brozman M, Jakubovský J (1976) Influence of fasting and stimulation on the rat gastric endocrine cells. Histochemical and ultrastructural study. Histochemistry 49(4): 315–325. https://doi.org/10.1007/BF00496135