Light-and electron-microscopic studies on the development of the cardiac region of stomach in prenatal rats

Mevlüt ASAR, Erdoğan KOCAMAZ, Necdet DEMİR, İsmail ÜSTÜNEL

Dept. of Histology and Embryology, Medical School of Akdeniz University, Antalya, TURKEY

In this study, the development of the cardiac of rat stomach was investigated by light and electron microscope. Thirty rat fetuses ranging between 17-21 days old were used for this purpose. In conclusion, we confirmed that on the 19th day of fetal period, indifferent epithelial cells differentiated partly; on the 20th day, stratified epithelium converted into simple columnar type, the glandular structure of the epithelium appeared, the strata of the cardiac wall were occurred completely; on the 21st day, all these structures were similar to those of adult rats. [Turk J Med Res 1996; 14(2):48-53]

Key Words: Rat, Stomach

The development of stomach was studied in different species as rat, mouse and man by various methods like TEM, SEM, histochemistry (1-19). However, we do not have much knowledge on the ultrastructural development of the stomach especially during fetal period. Moreover, most of these studies are focused on the gastric epithelium (1,3,7,10-12,19).

The aim of this study was to determine the epithelial cellular differentiation and the morphology and the time of the appearance of glands and strata of the cardiac wall by using light-and electron-microscope. Furthermore, we studied the relations between these structures by light microscope morphometrically.

MATERIALS AND METHODS

Thirty rat fetuses (6 fetuses in each group ranging from 17 to 21 days in age have been used in this study. The stomach was removed from rat fetuses. The cardiac regions of fetal stomach obtained were prefixed in 2% osmium tetroxide respectively and dehydrated in ethanol solutions, and embedded in araldit OY212. Semithin sections from araldit blocks were stained with 1% toluidine blue for light microscopy, with uranyl acetate and lead citrate its ultrathin sections for electron microscopy (20). Parameters studied were the epithelial cellular differentiation and thickness of different strata (epithelium, blastemic tissue, lamina propria, submucosa, tunica muscularis) of total wall, the height of the pit-gland structures, the appearance and morphology of these structures. Semithin and thin sections were evaluated by using light -and electron- microscope respectively. The thickness and height of these parameters were measured by micrometer on sections at the light microscopic level.

Statistical analysis was carried out using Student's t test.

Received: Dec. 20, 1995 **Accepted:** Sept. 3, 1996

Correspondence: Mevliit ASAR
Dept. of Histology and Embryology
Medicah School of Akdeniz University,
Antalya, TURKEY

RESULTS

Light microscopic observations: On the 17th and 18th days of fetal period, the epithelium was stratified, there were pit structures on its surface. On the 17th day, the mean thickness of the epithelium was the heighest of fetal period (62.200±2.191 urn). On the 19th day, it decreased to 38.660±3.797 urn ($p<0.05$) (Fig. 1,2,3, Table 1). On the 17th day, the blastemic tissue was rich in cells and vessels (Fig. 1). On the 17th day, the tunica muscularis composed of two layers. On the 18th day, the mean thickness of the tunica muscularis was 135.000±3.835 urn, and on the 19th day decreased to 77.333±2.291 urn ($p<0.0001$) (Fig. 1,2,3, Table 1). On the 17th and 18th days, the tunica serosa consisted of single layer with oval and rounded cells (Fig. 1,2). On the 20th day, the stratified epithelium of cardia converted partly into simple columnar type. The glandular structures and muscularis mucosa were seen in the epithelium and mesenchyme respectively (Fig. 4). On the 21st day, the wall of cardia was similar to those of adult rats (Fig. 5). On the 18th day, the mean thickness of total fundic wall was the heighest of fetal period (325.000±8.074 urn) (Fig. 2, Table 1). On

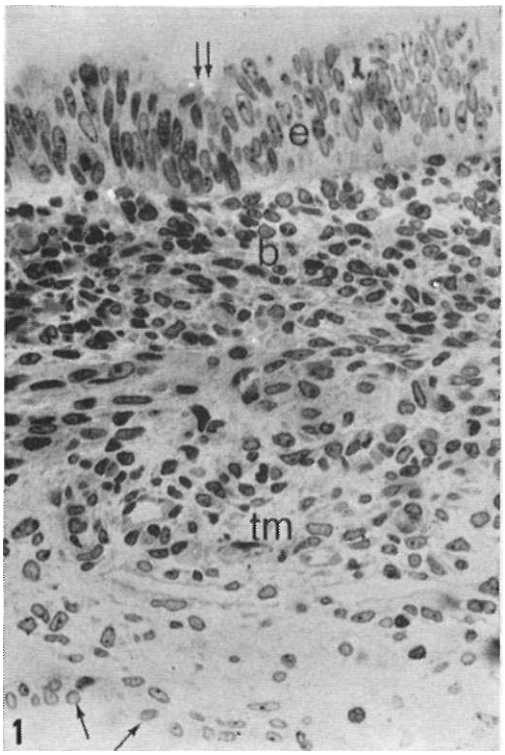


Figure 1. The cardia of rat stomach on the 17th day of fetal period. Epithelium (e), blastemic tissue (b), tunica muscularis (tm), tunica serosa (single arrows), primitive pit (double arrow). Semlthin section, toluidine blue, x320

the 20th day, the mean height of pit-gland was 73.760+2.006 urn, and 83.880±7.678 u.m on the 21st day.

Electron microscopic observations: On the 17th and 18th days of fetal period, the epithelium composed of ^differentiated cells. Nucleus occupied a large proportion of the cytoplasm. Free ribosomes were observed in the cytoplasm. Free ribosomes were observed in the cytoplasm. Mitochondria were oval and rounded in shape. The junctional complexes were seen between the lateral walls and microvili on the apical cell surface (Fig. 6,7). On the 19th day of fetal period, the pit structures of stratified epithelium protruded toward the mesenchyme to form the glandular structures as shown by TEM (Fig. 8). In this period, the epithelium of the pit structure converted into single columnar type, but the surface epithelium of cardia was still stratified. Surface and glandular-epithelial cells had microvilli on apical surface and secretory granules in apical cytoplasm (Fig. 8). On the 20th day of fetal period, the cardiac glands seemed in the epithelium. Surface- and glandular-epithelial cells had more microvilli, secretory granules and free ribosomes than those

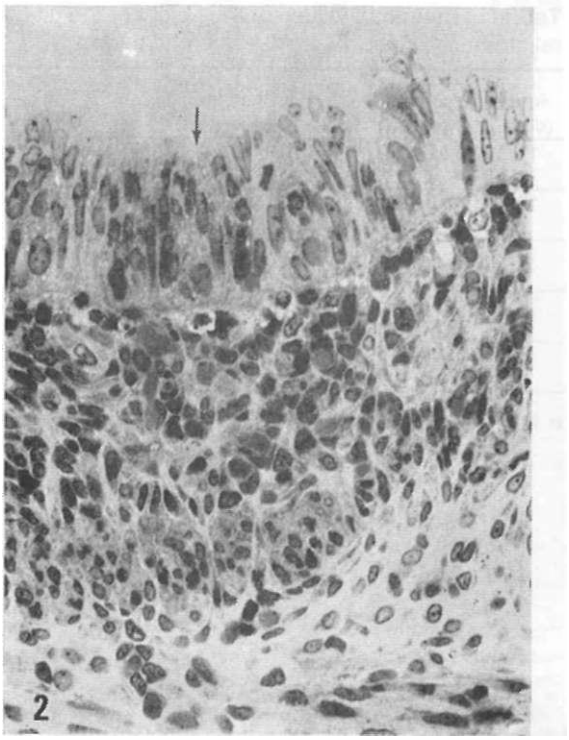
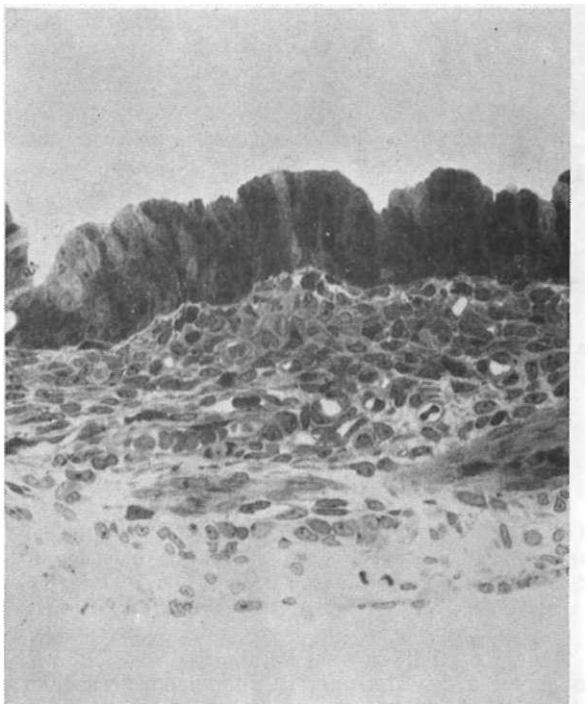


Figure 2. The developing pit (single arrow) in the cardia of rat stomach on the 18th day of fetal period. Semithin section, toluidine blue, x.320.



3

Figure 3. The histological structure of the cardia rat stomach on the 19th day of fetal period. Semithin section toluidine blue, x320

Table 1. The mean values of the height of the pit-gland and of the thickness of the cardiac wall and its strata of the rat stomach on the 17-21 days of fetal period.

Age (day)	Epithelium *(urn)*	Blastemic tissue (um)	Tunica muscularis (um)	Lamina propria (um)	Submucosa (um)	Total fundic wall (um)	pit-gland height (um)
17	62.200±2.191 a	72.667±1.607	135.167±2.47 e			303.33315.050	
18	62.017±3.686	78.667±2.492 c	135.000±3.835			325.00018.704 m	
19	38.660±3.797 b	69.500±1.026 d	77.333+2.291			196.66711.421 n	
20	29.200±0.800		66.667±0.637	96.50012.889 g	24.200±1.298 i	200.00013.163	73.76012.006 r
21	29.433±1.410		68.500±0.789	75.333±2.350 h	30.25±1.683 k	209.50018.391	83.88017.678 s

a, b: p<0.005; c, d: p<0.0001; e. f: p<0.0001; g, h: p<0.0001; i, k: p<0.05; m, n: p<0.0001; r, s: p>0.05

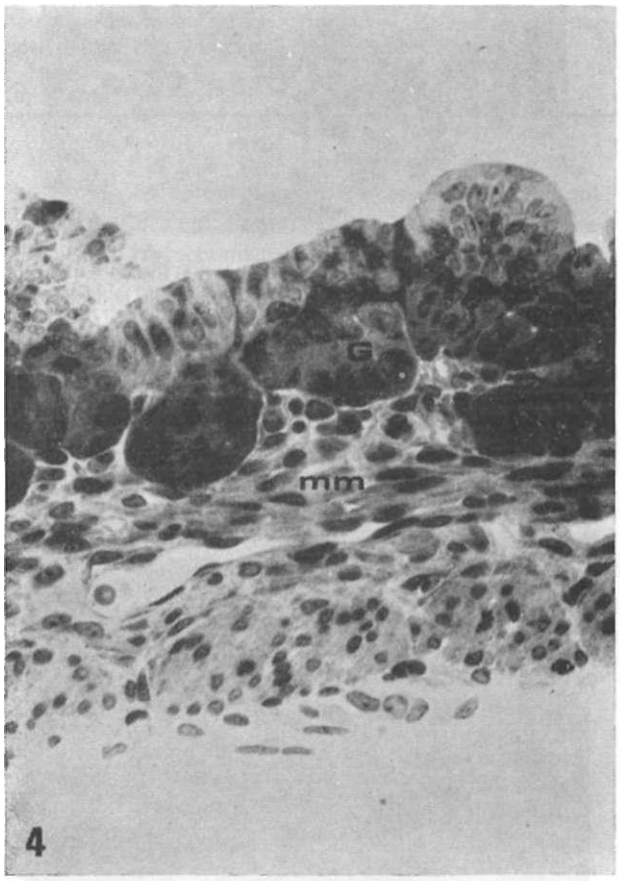


Figure 4. The cardia of rat stomach on the 20th day of fetal period. Glandular structure (G), muscularis mucosae (mm). Semithin section,Joluidine blue, x320.

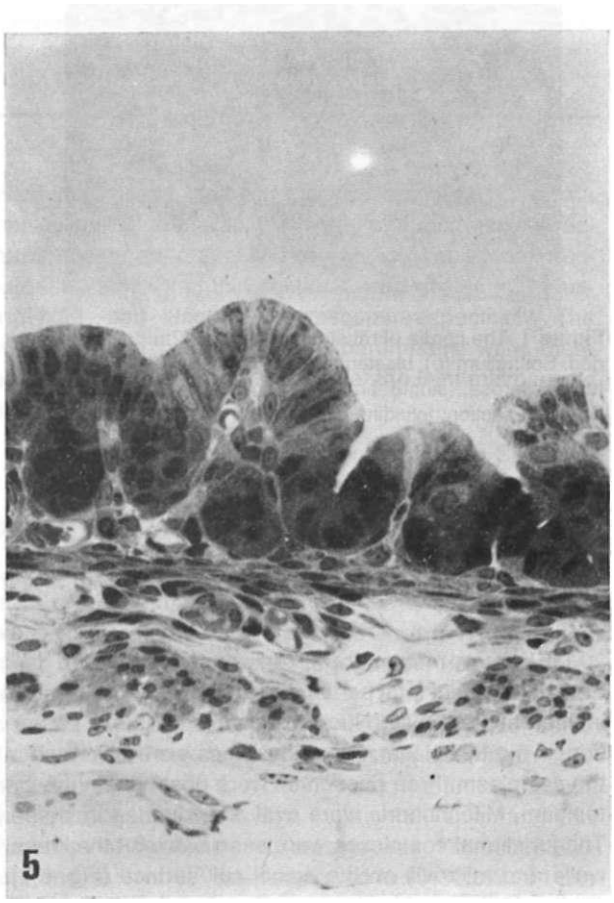


Figure 5. The differentiated glandular structures of the cardia of rat stomach on the 21st day of fetal period. Semithin section, toluidine bule, x320.

observed at previous periods. Endoplasmic reticulum expanded into the cytoplasm. The elongated nucleus was fixed in the basal cytoplasm of surface-and glandular cells (Fig. 9). On the 21st day of fetal period, the ultrastructure of surface-and glandular-cells of the epithelium differentiated well and interdigitations were quite extensive between the neighboring cells parallel to the developing events (Fig. 10).

DISCUSSION

It was reported that the striking changes of the developing stomach occur in the epithelium, mesenchyme and

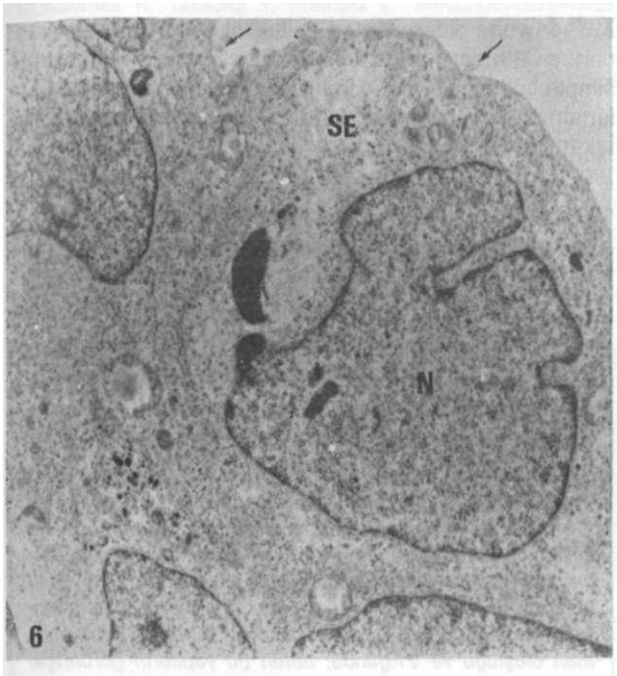


Figure 6. The electron micrograph of the surface epithelial cell (E) in the cardia of rat stomach on the 17th day of fetal period. Nucleus (N), microvillus (single arrows), x4500

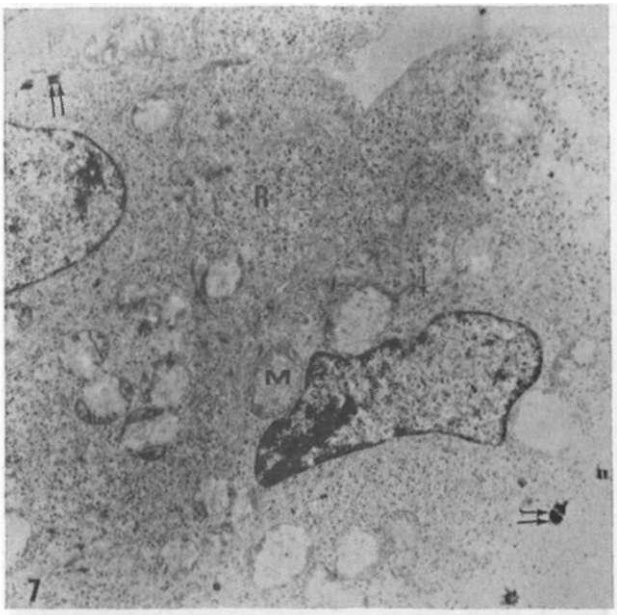


Figure 7. The electron micrograph of the cardia of rat stomach on the 19th day of fetal period. Microvillus (arrow heads), secretory granule (single arrow), basement membrane (double arrow) - x1350

the muscle layer (1-7) in fetal period and these findings are supported by our morphological findings. These events may be closely related to the development of the different layer of the muscle wall, as well as the formation of connective tissue elements and blood vessels. Herbert et al, reported that on the 19th day of fetal rat, the stratified epithelium of stomach changed to simple columnar

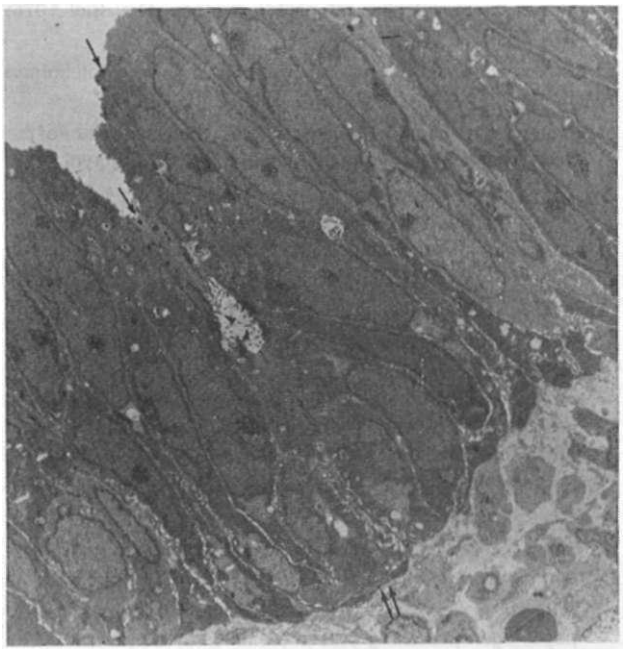


Figure 8. The electron micrograph of the glandular epithelial cell in **the** cardia of rat stomach on the 20th day of fetal period. Glandular structure (G), endoplasmic reticulum (single arrow), secretory granule (double arrows), x1350

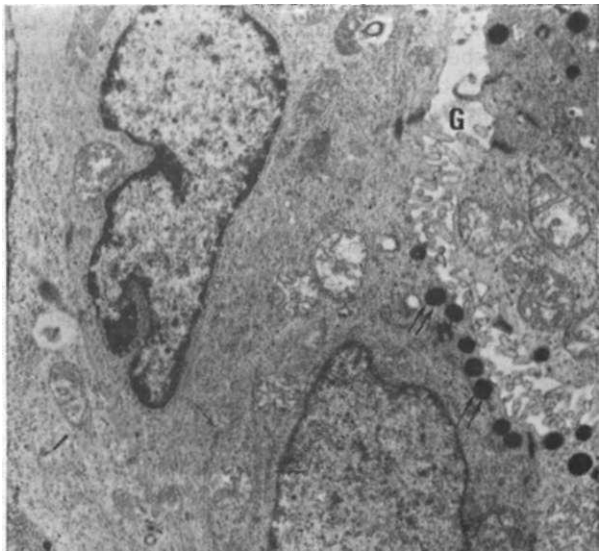


Figure 9. The electron micrograph of the glandular epithelial cell in the cardia of rat stomach on the 20th day of fetal period. Glandular structure (G), endoplasmic reticulum (single arrow), secretory granule (double arrow), x4500

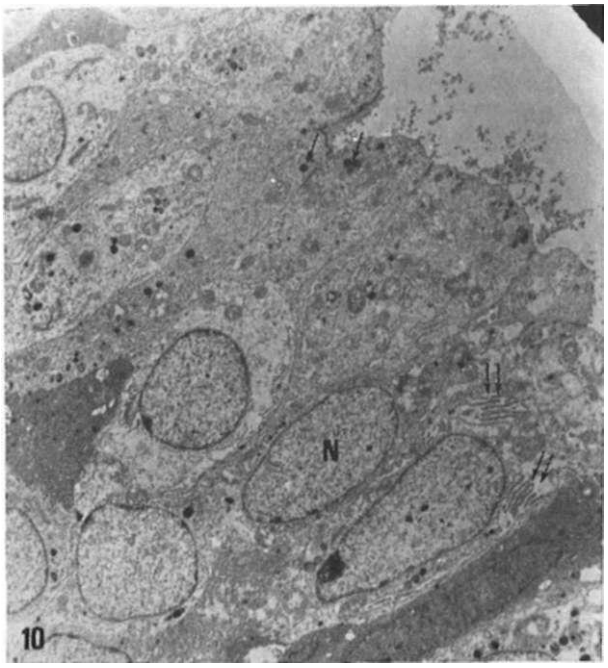


Figure 10. The electron micrograph of the cardia of rat stomach on the 21st day of fetal period. Nucleus (N), secretory granule (single arrows), interdigitation (double arrows) x1800

type, the glandular structures appeared and secretory granules occurred in the surface-and glandular-epithelial cell (1). Herbert's findings, related to secretory granules, is supported by our morphological findings as shown TEM (Fig. 8). We have seen that on the 20th day, the cardiac epithelium changed to simple columnar type completely, the glandular structures occurred and these findings were in accordance with those reported by Yeoman and Trier (2). On the 20th day, the appearance of the glandular structures can be explained by an intense epithelial cell proliferation. Interestingly, it was reported that the mitotic indices reached to a peak at the 20th day along with the appearance of the glandular structures (1,2). Unlike some investigators, we observed the structures of the microvilli on the 17th they but not 19th day. From 19th day to 20th day, the repeated increasing thickness of the cardiac wall can be related to the increasing surface of the cardiac epithelium and the forming glandular structures. We supported that in the last day of gestation, the lateral cell borders exhibited interdigitatidns with neighboring cells, and this appearance coincides with the period of rapid differentiation of epithelial cells as reported before (1,2,3). Accurate timing and sequence of the development of these structures may provide clue to understanding cellular interactions between the developing layers of the cardia. According to the findings of present studies, in fetal rat stomach it can be say: On the 19th day, the differentiated epithelial cell appeared first; on the 20th day, the stratified epithelium changed to the simple columnar type with lowest thickness, the glandular structure occurred and the strata of cardiac wall were taken form.

In conclusion, we confirmed that in fetal period of 19-21 days, the histological differentiation and maturation of the gastric cardia can be completed mostly at the light- and electron-microscopic level.

Prenatal sıçanda mide kardiya bölgesinin gelişmesi üzerine ışık ve elektron mikroskobik bir çalışma

Bu çalışmada, embriyonal 17-21.günler arasında sıçan midesi kardiya bölgesinin gelişmesi ışık ve elektron mikroskobu ile araştırıldı. Bu amaç için 30 sıçan embriyonu kullanıldı. Sonuçta, embriyonal 19.günde; indifferensiye epitel hücrelerinin kısmen farklılaştığı, 20.günde; çok katlı mukoza epitelinin tek katlı yapıya değiştiği, epitelde bez yapılarının görüldüğü, duvarın histolojik tabakalarının tamamen oluştuğu ve 21.günde; bütün bu yapıların görünüşte ergindeki yapılara benzedikleri saptandı. [Türk J Med Res 1996; 14(2):48-53]

REFERENCES

1. Herbert F, Helander MD. Ultrastructure and function of gastric mucoid and zymogen cell in the rat during development. Gastroenterol 1969; 56:53-70.
2. Yeomans ND, Trier JS. Epithelial cell proliferation and migration in the developing rat gastric mucosa. Dev Biol 1976; 53:206-16.
3. Menard D, Arsenault P. Cell proliferation in developing human stomach. Anat Embryol 1990; 182:509-16.
4. Otani H, Yoneyama T, Hashimato R, et al. Ultrastructure of the developing stomach in human embryos. Anat Embryol 1993; 187:145-51.
5. Franco A, Robina A, Guillen MT, et al. Histomorphometric analysis of the abomasum of the sheep during development. Ann Anat 1993; 175:119-25.
6. Acuff ME, Krause WJ, Cutt JH. The cardiac, oxyntic and pyloric glands in the developing opossum. Anat Ann 1989; 169:267-71.
7. Helander HF. Ultrastructure and function of gastric parietal cells in the rat during development. Gastroenterol 1969; 56:35-52.
8. Johnson LR. Functional development of the stomach. Ann Rev Physiol 1985; 44:199-215.
9. Deren JS. Development of structure and function in the fetal and newborn stomach. Am Clin Nutr 1971; 24:144-59.
10. Furihata O, Iwasaki Y, Sugimura T, et al. Differentiation of pepsinogen-procuding cells in the fundic and pyloric mucosa of developing rats. Cell differentiation 1973; 189:179-89.
H. Ekelund M, Hakanson R, Hedenbro J, et al. Endocrine cells and parietal cells in the stomach of the developing rat. Acta Physiol Scand 1985; 124:483-97.

12. Kataoka K, Takeoka Y, Furihata C. Immunocytochemical study of pepsinogen 1-produsing cells in the fundic mucosa of the stomach in developing mice. Cell Tissue Res 1990; 261:211-7.

13. Nomura Y. On the submicroscopic morphogenesis parietal cell in the gastric gland of the human foetus. Z Anat Entwicklungsgesch 1966; 125:316-22.

14. Stein BA, Buchan AMJ, Morris J, et al. The ontogeny of regulatory peptide-containing cells in the human stomach. Anat Embryol 1983; 182:509-16.

15. Facer P, Bishop AE, Cole GA, et al. Development profile of chromogranin, hormonal peptide and 5-hydroxytryptamine in gastrointestinal endocrine cells. Gastroenterol 1989; 97:48-57.

16. Fath El-Bab MR, Schwartz R, AN AMA. Morphological studies on the stomach of sheep during prenatal life. Zbl Vet Med C Anat Histol Embryol 1983; 12:139-53.

17. 0nolfo JP, Lehy T. Comparative development of gastrin and somatostatin cell populations in the pancreas, stomach, and duodenum of the rat during the perinatal period. Anat Rec 1987; 218:416-25.

18. Krause WJ, Yamada J, Cutt JH, et al. An immunohistochemical survey of gastric proteinase (pepsinogen and prochymosin)-containing cells in the developing opossum (Didelphis virginiana). J Anat 1987; 154:259-63.

19. Lipkin M. Growth and development of gastrointestinal cells. Ann Rev Physiol 1985; 47:175-97.

20. Bozzola JJ, Russel LD. Electron Microscopy Principles and Techniques for Biologists: Boston (USA): Jones&Barltlett Pub Inc, 1992:15-38.