J. Expt. & Clin. Anat. 5(1): 48-58(2006).

JECA

Histological and Morphometric Studies on the Age-Associated Changes in the Colon of the Mouse

M.A. MOTABAGANI

Department of Anatomy, College of Medicine, King Faisal University, Dammam, Saudi Arabia

ABSTRACT

The present work was designed to study the morphological and morphometric age-associated changes in the mouse colon in order to explore any changes that might lead to colon dysfunction.

Forty mice were used in this study. They were divided equally into four groups aged 1, 2, 12 and 24 months. Light microscopy and scanning and transmission electron microscopic examinations of the colon were conducted. Measurements of the outer diameter of the colon, the total wall thickness, the crypt's length and the muscle coat thickness were carried out. Statistical analyses of the results were done.

In old aged mice (12 and 24 months) there was apparent decrease in the number of goblet cells and marked increase in the amount of collagenous fibers in the mucosa and submucosa compared to the young aged mice (1 and 2 months). Statistical analysis showed a significant increase in the outer diameter and the length of the crypts in old aged mice. In addition a significant decrease in the nerve cell number of Auerbach's plexus was also recorded. Using TEM there was an apparent reduction in both the striated border and the intercellular spaces in the mucosal epithelium of the old mice. In addition there was a marked increase in the undifferentiated stem cells at the bases of the crypts. Using SEM many areas of denuded epithelium were also observed in old mice.

The remarkable decrease in (1) the number of mucosal goblet cells and microvillar and intricate systems, (2) the number of neurons and interstitial cells of Cajal in the myenteric plexus, (3) the muscle wall thickness, with the observed increase in deposition of collagen fibers in the mucosa and submucosa of old-aged mice colon probably contribute to colon dysfunction in elderly.

Key words: Colonic mucosa, goblet cells, colonic crypts, collagen, Auerbach's plexus.

Advancing age tends to be accompanied by predictable changes in the structure and function of different organs (Watters et al., 1990). Chronic constipation is one of the most common digestive complains in the elderly. It affects as many as 26 percent of elderly men and 34 percent of elderly women (Schaefer and Cheskin, 1998).

Colorectal physiologic and psychologic dysfunctions are common in elderly constipated subjects (Merkel et al., 1993). The significant risk factors in old women were failure of the anorectal angle to open or disturbed pelvic floor function and rectal evacuation (Camilleri et al., 2000).

Since the enteric nervous system (ENS) is responsible for coordinating and integrating the motility of the gut (El-Salhy et al., 1999; Furness and Costa, 1987) ,it may possibly play a crucial role in the pathophysiology of colorectal motility disorders in old age (Wedel et al., 1999, 2002).Abnormally increased number of myenteric ganglia in the colon of aging population has been also considered a contributing factor (Hanani et al.,2004).

The aim of the present work is to investigate the morphological and morphometric age-associated changes in the mouse colon in order to explore any changes that might lead to organ dysfunction.

MATERIAL AND METHODS

Forty Swiss mice were used in this study. The animals were divided into four groups, ten mice each. The first group aged 1 month, the second group aged 2 months, the third group aged 12 months and the fourth group aged 24 months. The animals were sacrificed and the anterior abdominal wall was opened by a midline incision to explore the whole large intestine. One cm annular specimens were excised from the distal colon and washed several times with normal saline.

For light microscopy, specimens were fixed in 10% formol saline and processed to obtain 7 μm thick paraffin sections. The sections were stained with haematoxylin and eosin (H & E),

periodic acid-Schiff (PAS) reaction and Masson's trichrome technique (Bancroft and Gamble, 2001).

For scanning electron microscopic study, 3 5 mm colon specimens were cut and fixed in 2% paraformaldehyde and 2-5% glutaraldehyde in 0.1 M phosphate buffer for 24 hours. Fixation and washing were carried out at 4°C; the tissues were then dehydrated through ascending grades of ethanol and substituted with isomylacetate. The colonic specimens were dried at critical point using liquid CO_2. The tissue specimens were mounted on stubs with aluminum conducting tape and coated with 20 nm layer of gold in JFC ion sputterer (Glauret, 1986; Meek, 1970; Robinson et al., 1997). The specimens were examined with XL 30 scanning electron microscope (Philips) operated 30 KV (Robinson et al., 1997). The luminal surface of the colon was examined and photographed.

For transmission electron microscopy, specimens were fixed in 1% glutaraldehyde and then cut into small pieces. Samples were then fixed in 2.5% glutaraldehyde, for 24 hours and then in 1% osmium tetroxide for 1 hour. After dehydration in ethanol and propylene oxide, specimens were embedded in resin. Semithin sections were cut and stained with toluidine blue and examined by light microscopy for orientation. Ultrathin sections were stained in a saturated solution of uranyl acetate for 30 minutes followed by lead citrate for 5 minutes, and examined and photographed with transmission electron microscope (Philips).

Statistical analysis:

Ten specimens were examined from each age group and ten measurements were taken from each specimen. The measurements included the following: The outer diameter of the colon; the total wall thickness measured from the serosa to the base of the crypt (excluding the crypt length); the crypt length (height) measured from the base of the crypt to the surface epithelium; the muscle coat thickness including the inner circular and the outer longitudinal coats; the number of neurons in Auerbach's plexus was counted per 10 fields x 200 magnifications in each group (Collure and Hameer, 1996) using Zeiss integrating eyepiece. The measurements figures were calculated and processed for statistical analysis and comparison of means using ANOVA test, SPSS program (Altman, 1994).

RESULTS

1. Light Microscopic Results:

1.1 Young mice (groups 1 and 2):

The colonic mucosa of young mice was thrown into numerous crescentic folds, the plicae semilunares (Fig. 1A). It consisted of the surface epithelium, intestinal crypts, lamina propria and muscularis mucosae. The surface epithelium was formed of closely packed tall columnar cells with basal oval nuclei interposed with occasional goblet cells. The crypts appeared as closely packed simple tubular glands occupying the thickness of the lamina propria. The cell population of the crypts was composed of numerous goblet cells (Fig. 1B). Surface epithelial cells as well as undifferentiated stem cells were also present. The brush border of the surface columnar cells as well as the goblet cells in the upper two-thirds of the crypts exhibited moderately positive PAS reaction. However, the epithelial cells at the lower portion of the crypts appeared relatively smaller in size, and showed either weak positive or negative PAS reaction (Fig 1C). The lamina propria was densely cellular, housing many lymphoid cells (Fig 1B). The muscularis mucosae appeared as a well defined layer, which was closely applied to the bases of the crypts (Figs. 1D). Both the lamina propria and the submucosa showed minimal amount of collagenous fibers observed in sections stained with Masson's trichrome stain (Fig. 1B). A well developed muscularis externa was evident. It was formed of an inner circular and an outer longitudinal smooth muscle layers. The circular muscle layer was relatively thicker than the longitudinal one (Figs. 1B&E). Examination of the Holm's silver stained sections showed the presence of submucosal (Meissner's) plexus of nerves. The nerve cells were stellate-shaped with vesicular nuclei (Figs. 1D). The myenteric (Auerbach's) plexus was seen enclosed between the circular and longitudinal muscle layers of muscularis externa. It consisted of densely packed neurons and glial cells (Fig. 1E).

1.2 Old Mice (groups 3 and 4):

Examination of the old mice colon showed an apparent increase in the outer diameter and in the length of the mucosal folds as well as in the number of the mucosal crypts associated with narrowing of the lumen (Fig. 2A). The distribution of both the columnar and goblet cells was similar to that of the young age groups (Fig. 2B&C). In PAS stained sections, the goblet cells appeared fewer and intensely stained as compared with the young age groups (Fig. 2D). The lamina propria was more heavily infiltrated with lymphoid cells (Fig.2C). The muscularis mucosa was relatively thick but occasionally disrupted by scattered areas of dense lymphocytic infiltration. Marked increase in the amount of collagenous fibers and many dilated blood vessels were observed in both the lamina propria and submucosa (Figs. 2B&C).. Examination of the myenteric plexus between the two muscle layers of musculosa externa showed an apparent decrease in the nerve cells and supporting glial cells (Fig. 2E).

2. Scanning Electron Microscopic Results:

2.1 Young mice (groups 1 and 2):

Scanning electron microscopic examination at this age revealed that the luminal surface of the colon showed regularly spaced crypt openings surrounded by an intact layer of surface epithelial cells (Fig. 1F). Mucous secretion was observed in the openings of the crypts. The surfaces of the cells adjacent to the crypt opening were flat, whereas the surfaces of the cells at the intercryptal areas were rounded and bulging over the surface (Fig. 1F).

2.2 Old mice (groups 3 and 4):

Scanning electron microscopic examination at this stage, revealed the presence of many black spots on the luminal surface (Fig. 2F). The mucous secretion of the goblet cells was relatively less than that observed in the young age. Most of the surface epithelial cells were flat and the rounded epithelial cells in the intercryptal areas were less frequently seen .Moreover, many areas denuded of epithelium as well as areas with no epithelial covering were also observed.

3.Transmission Electron Microscopic Results:

3.1 Young mice (groups 1 and 2):

The epithelium of the colonic mucosa consisted of a single layer of cells. The most numerous cells present were the absorptive cells and the mucous-secreting goblet cells. The former predominated in the surface epithelium while the latter was the most numerous in the colonic crypts (Fig. 1G and 1H). Small undifferentiated stem cells were confined to the bases of the crypts, as well as cells shoeing signs of apoptosis and necrosis (Fig 1I). The absorptive cells appeared tall and rectangular in profile with the long axis perpendicular to the basal lamina. The nuclei were either basal or central and elongated vertically. The goblet cell extended from the basal lamina to the free surface. It had a narrow base containing the nucleus with a large peripheral nucleolus. The apical cytoplasm often appeared distended and occupied by mucinogen secretory granules.

The apical surfaces of the epithelial cells lining the lumen possessed well-developed, closely packed parallel microvilli, giving the apical surface a striated appearance, the brush border (Fig.1G). The intercellular spaces between the epithelial cells were wide and contained cellular processes extending from the lateral plasma membranes of the cells to interdigitate with similar processes of adjacent cells, forming an intricate system (Fig. 1H).

The myenteric ganglia were composed of neurons and glial cells surrounded by a continuous basal lamina and collagen fibrils (Fig.1J).Multiple intramuscular unmyelinated axonal terminals containing mitochondria and synaptic vesides of both granular and agranular type were observed(Fig.1K). Few myelinated profiles were also present. Interposed between the enteric neurons and nerve fibers were numerous interstitial cells of Cajal (Fig.1L). These sells resembled fibroblasts and showed numerous gab junctions with each other

3.2 Old mice (groups 3 and 4):

In this group the apical surfaces of the epithelial cells lining the lumen possessed few poorly-developed, loosely packed microvilli (Fig. 2 G). The intercellular spaces between the epithelial cells lining the surface and the crypts were narrow and small. Only few cytoplasmic processes extended from the lateral cell

membranes into the intercellular space (Fig. 2H). At the bases of the crypts were numerous undifferentiated stem cells as well as many oligomucous cells (Fig. 2I).

The myenteric ganglia contained only few glial cells and neurons (Fig. 2J). The interstitial cells of Cajal were not evident in all sections examined. The smooth myofibers of the muscularis externa were widely separated by collagen fibers (Fig.2J).

4. Statistical Results:

There was a significant increase in the outer diameter of the colon with age ($P < 0.01$). However, the total wall thickness was greater at 2 months-old mice then it declines significantly with age ($P < 0.05$). The same results were noticed for the thickness of the muscle coat. However, the length of the crypts increased significantly with age ($P < 0.01$) (Table 1 and Graph 1).

There was a significant increase in the number of cells of Auerbach's plexus from one month-old mice to two months-old mice then the number declines significantly with age($P < 0.05$) (Table 2 and Graph 2).

TABLE (1): The different measurements (mean ± SD) in the transverse sections of mice colon

The measures in (M)	One month old	Two months old	One year old	Two year old	Probability of significance
he outer diameter	1900 ± 60	2400 ± 80	2900 ± 85	3200 ± 88	< 0.01
he total wall ickness [excluding ie crypt length]	300 ± 58	470 ± 60	490 ± 35	350 ± 54	< 0.05
he crypt length	460 ± 28	650 ± 35	850 ± 40	990 ± 85	< 0.01
The muscle coat thickness	100 ± 15	220 ± 40	190 ± 30	160 ± 40	< 0.05

TABLE (2): The mean number of nerve cells of Auerbach's plexus/ certain visual field in mice colon in different age groups

	One month old	Two months old	One year old	Two years old	Probability of significance
The mean number of nerve cells of Auerbach's plexus/ certain field	12.75 ± 1.4	17.52 ± 2.1	9.85 ± 3.78	7.75 ± 4.5	< 0.05

DISCUSSION

In the present study, the mucus-secreting goblet cells appeared to predominate in the crypts whereas the luminal surface was almost entirely lined by columnar epithelial cells. Goblet cells lining the upper part of the crypts had a moderate to intense positive PAS reaction while those near the bases of the crypts showed either weak positive or a negative PAS reaction. The later cells may represent the oligo-mucous cells, the precursors of the goblet cells or the vaculated cells which were considered to be the stem cells of the colonic epithelium (Cormack, 1987).Goblet cells pass through a single secretory cycle. They synthesize their mucin in the crypts and migrate upward from their site of origin in the crypts to the site of extrusion on the luminal surface where

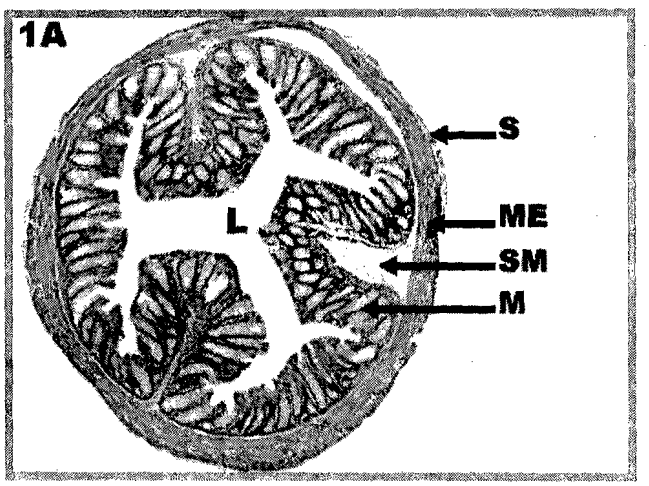


Fig. 1A: Photomicrograph of a complete transverse section of a young mouse colon showing the four layers characteristic of the alimentary canal, namely, the mucosa (M), the submucosa (SM), the muscularis externa (ME) and the serosa (S). The mucosa is thrown into numerous crescentic folds projecting into the lumen (L). Paraffin section; H&E; X40.

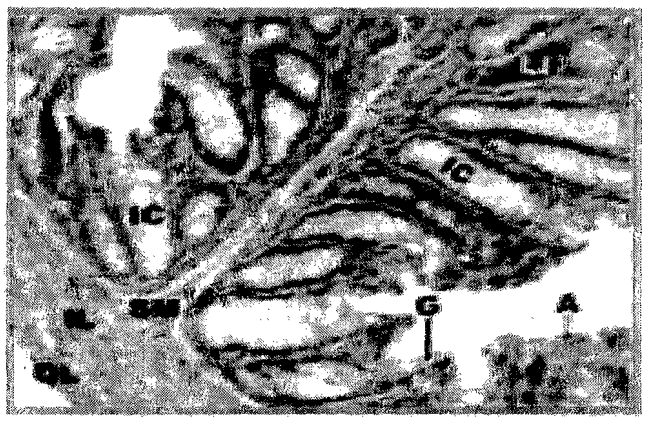


Fig. 1B: showing the mucosal surface lined by simple epithelium consisting of absorptive cells (A) and goblet cells (G). The straight tubular intestinal glands (IC), crypts of Lieberkühn, contain numerous goblet cells. . The lamina propria (LP) is highly cellular, housing many lymphoid cells (LI). Little amount of collagenous fibers (blue) deposited in the submucosa (SM) and in the interstitial tissue of the lamina propria between the colonic crypts. The inner circular (IL) and the outer longitudinal (OL) smooth muscle layers of the muscularis externa are indicated. Paraffin section; H&E; X200.

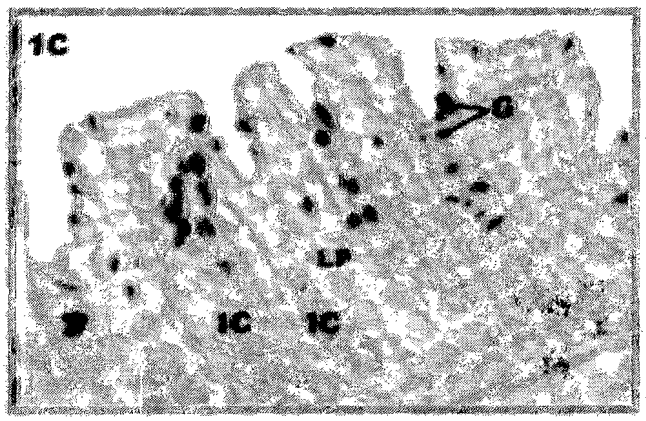


Fig. 1C: showing the intestinal crypts (IC) embedded in the lamina propria (LP) of the mucosa. The goblet cells (G) in the upper two-thirds of the crypts exhibit a moderate PAS reaction while cells in the lower third show weak positive or negative reaction. Paraffin section; PAS reaction; X100.

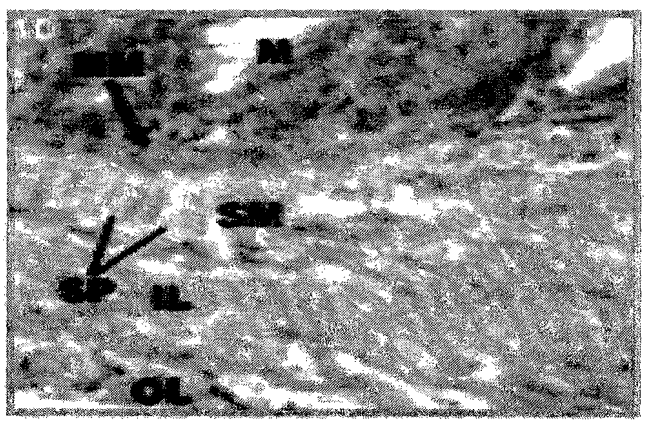


Fig. 1D: showing the submucosal plexus of nerves (SP) embedded in the submucosa (SM) between the muscularis mucosae (MM) and the inner circular layer (IL) of the muscularis externa. Paraffin section; Masson's trichrome technique; X200.

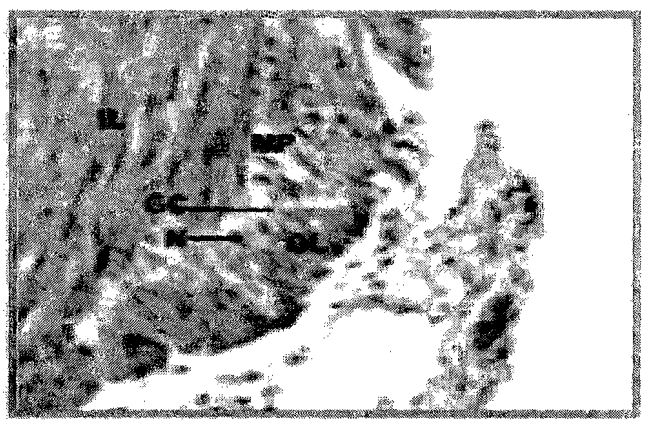


Fig. 1E: showing the myenteric plexus (MP) enclosed between the inner circular (IL) and the outer longitudinal (OL) layers of the muscularis externa. The plexus houses a group of densely packed neurons (N) with large nuclei and glial cells (GC) with small nuclei. Paraffin section; Masson's trichrome technique; X640.

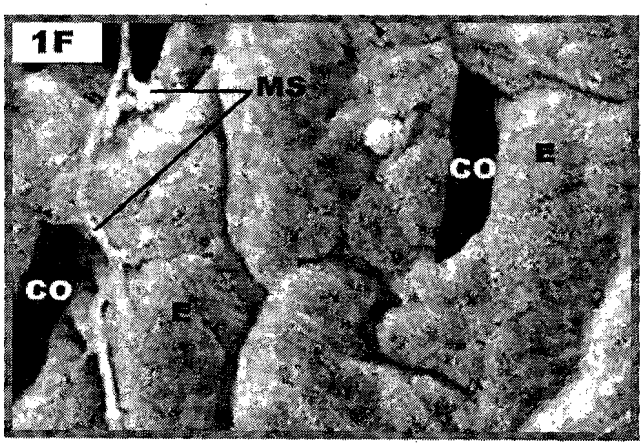


Fig. 1F: High -power scanning electron micrograph of the luminal surface of young mouse colon showing the epithelial cells (E) and the cryptal openings (CO). Epithelial cells close to the cryptal openings are flat while those covering the intercryptal areas are rounded and bulging cells. Mucous secretion (MS) is observed arising from mouths of the crypts and covering the mucosal surface. X800.

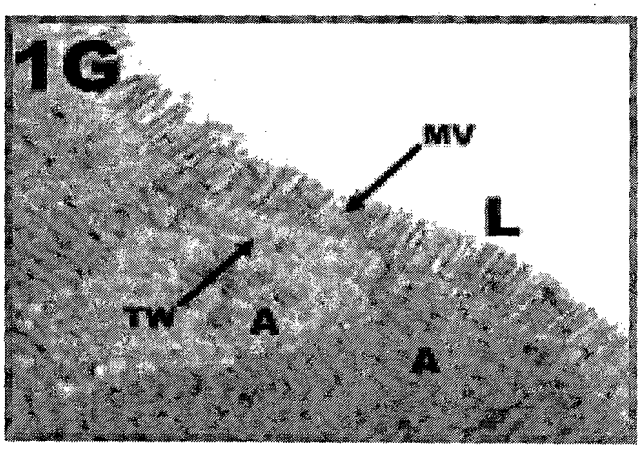


Fig. 1G: spans the surface absorptive cells (A) lining the lumen (L). The luminal cell membranes have well-developed, closely packed microvilli (MV). The filaments of the microvilli insert into a terminal web (TW).

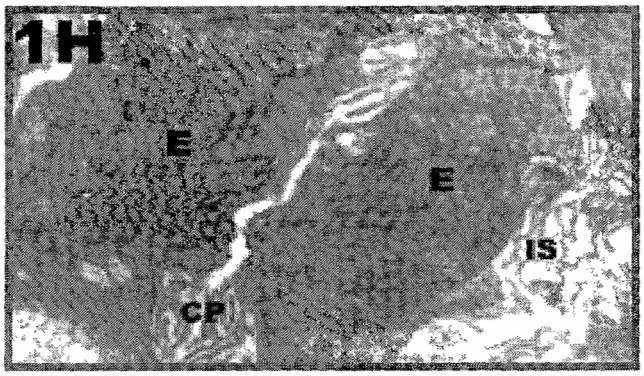


Fig. 1 H: shows the basal portions of adjacent epithelial cells (E) in the upper one-third of the crypt. The intercellular space (IS) is wide and occupied by interdigitating cytoplasmic processes and membrane leaflets (CP).

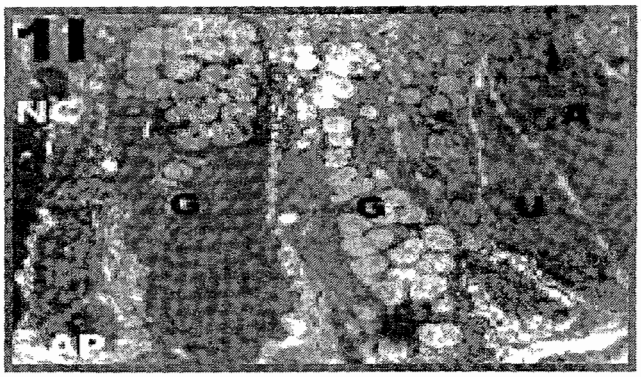


Fig. 1 I: shows the lower one-third of the colonic crypt. The cells present include goblet cells (G), absorptive cells (A) and undifferentiated stem cells (U). Also present in the epithelium are cells with electron-dense cytoplasm (NC) and cells with condensed fragmented nucleus (AP).

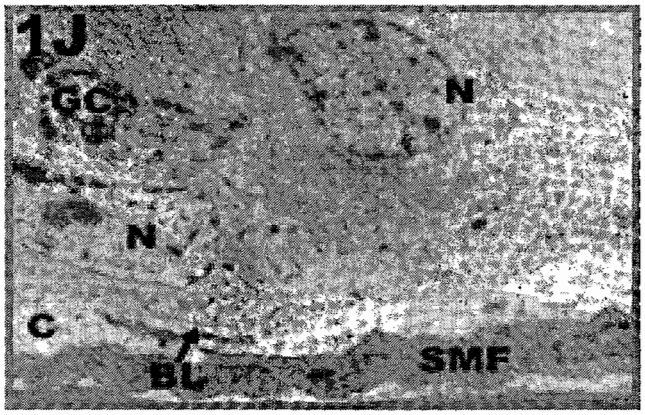


Fig. 1 J: spans a portion of a myenteric ganglion with multiple unmyelinated axons (UA), their axoplasm contains mitochondria (MT) and synaptic vesicles of both granular (GV) and agranular (AV) types.

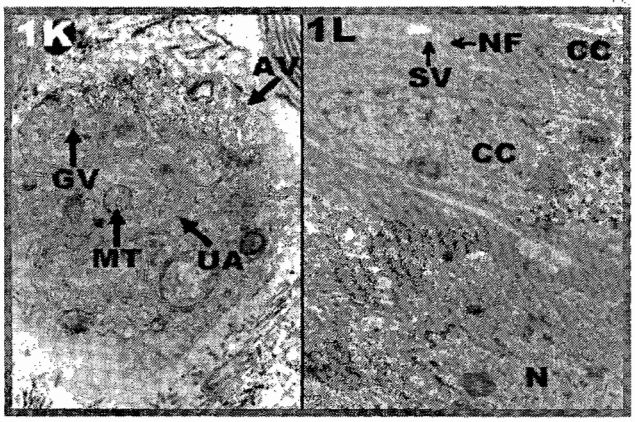


Fig. 1K: shows neurons (N) with neuronal extensions and a glial cell (GC). The neuron has a large perikaryon and its nucleus has an extended chromatin and a prominent nucleolus. The ganglion is surrounded by a continuous basal lamina (BL) and collagen fibrils (C). Smooth myofibers (SMF) of the muscularis externa are seen sectioned longitudinally.

Fig. 1L: This field exhibits the interstitial cells of Cajal (CC) interposed between the enteric neurons (N) and in close contact with nerve fibers (NF) containing synaptic vesicles (SV). These cells resemble fibroblasts and show numerous gap junctions with each other.

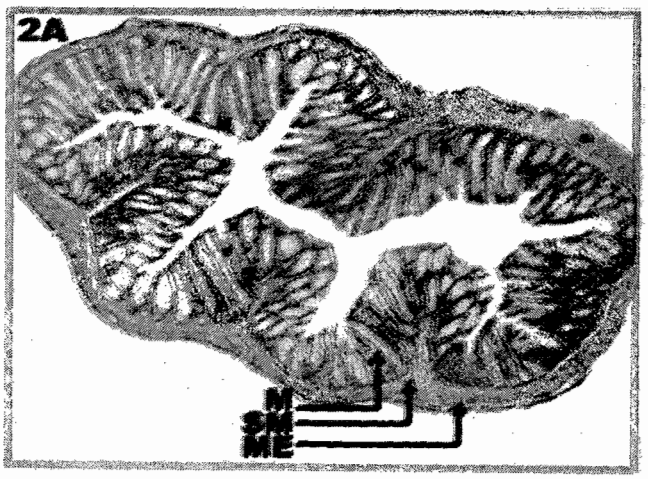


Fig. 2A: Photomicrograph of a complete transverse section of an old mouse colon showing the mucosa (M), the submucosa (SM), and the muscularis externa (ME). There is an apparent increase in the outer diameter, in the length of the mucosal folds and in the number of intestinal crypts. Paraffin section; H&E; X40.

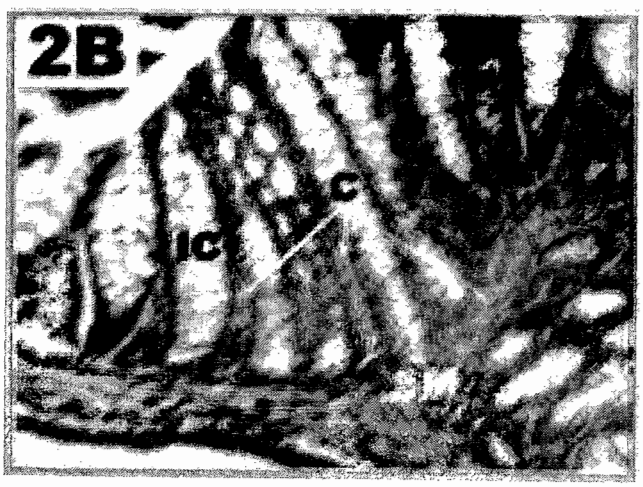


Fig. 2B: shows heavy deposition of collagenous fibers (C) in the submucosa (SM) and in the interstitial tissue of the lamina propria between the intestinal crypts (IC).

Fig. 2C: shows dense lymphocytic infiltration (LI) present in the lamina propria. Many dilated blood vessels (BV) are seen beneath the bases of the intestinal crypts (IC) and in the submucosa (SM). Paraffin section; Masson's trichrome technique; X200.

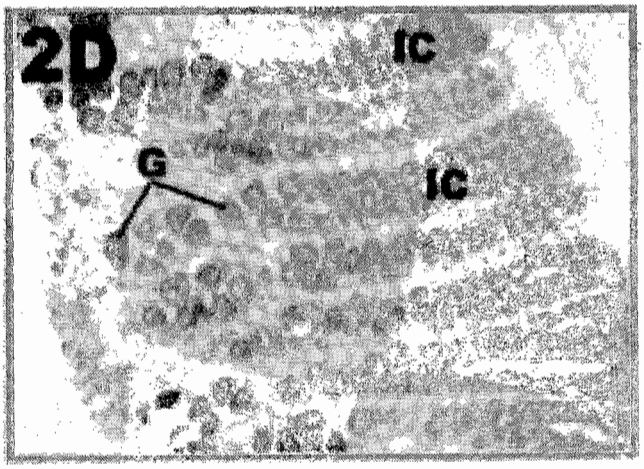


Fig. 2D: shows intense positive PAS reaction of the goblet cells (G) in the intestinal crypts(IC) although their number is relatively decreased. Paraffin section; PAS reaction; X400.

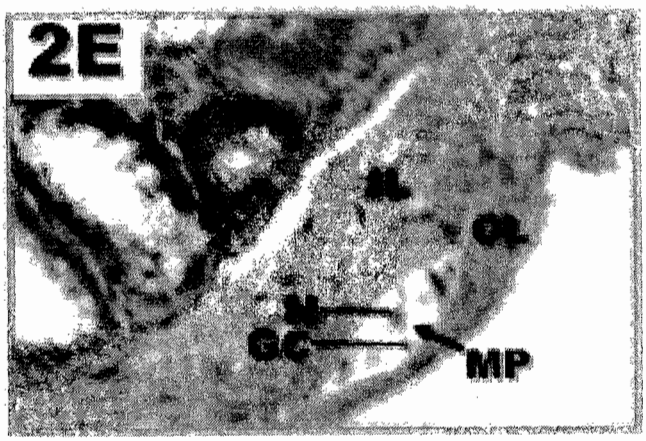


Fig 2E: shows a marked decrease in both the nerve cells (N) and the glial cells (GC) of the myenteric plexus (MP) situated between the inner circular (IL) and the outer longitudinal (OL) layers of the muscularis externa. Paraffin section; Masson's trichrome technique; X640.

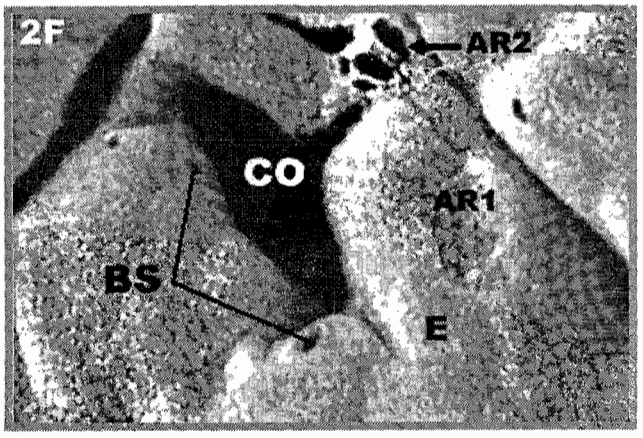


Fig. 2F: Scanning electron micrograph of the luminal surface of an old mouse colon showing the presence of many black spots (BS). Most of the epithelial cells (E) are flat. The crypt openings (CO) have little or no mucous secretion. Areas of denuded epithelium (AR1) as well as areas with no epithelial covering (AR2) are present on the luminal surface. X1200.

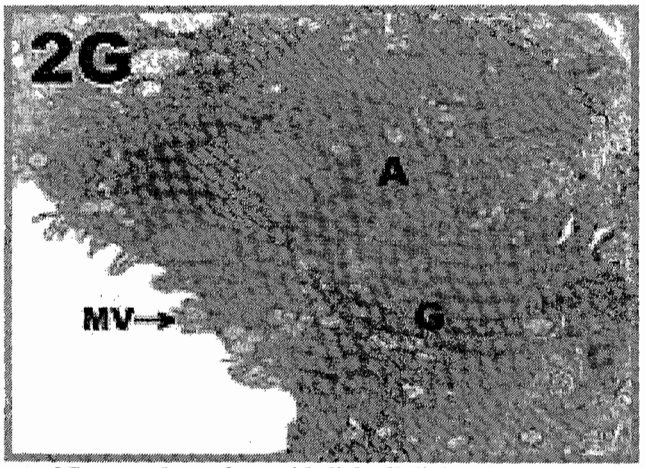


Fig. 2G: spans the surface epithelial cells lining the lumen. The luminal cell membranes of both the absorptive (A) and goblet (G) cells have poorly-developed, loosely packed microvilli (MV).

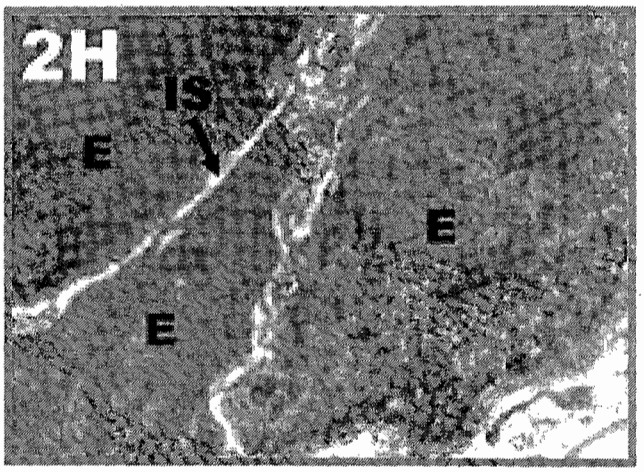


Fig. 2H: shows the basal portions of adjacent epithelial cells (E) in the upper one-third of the crypt. Few cytoplasmic processes extend from the lateral cell membranes into a narrow intercellular space (IS).

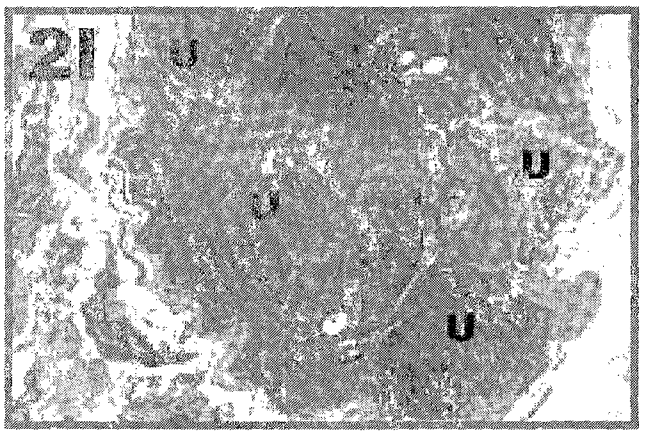


Fig. 2I: shows the lower one-third of the crypt containing multiple undifferentiated stem cells (U).

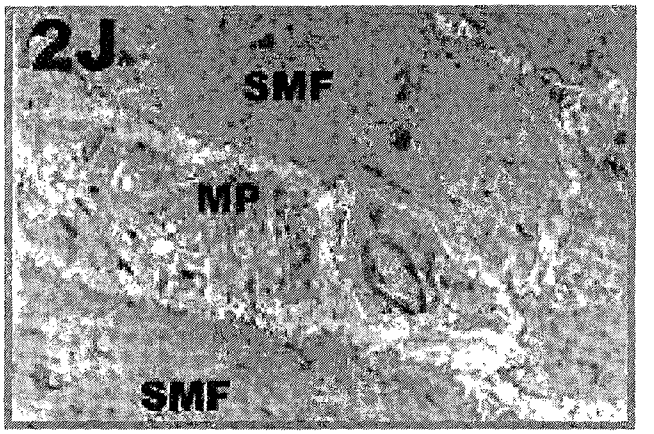
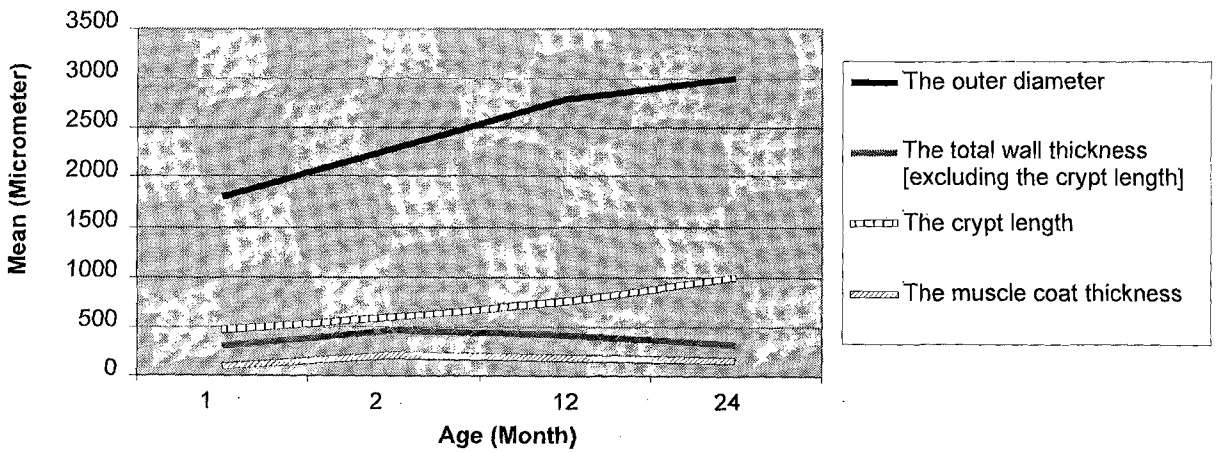
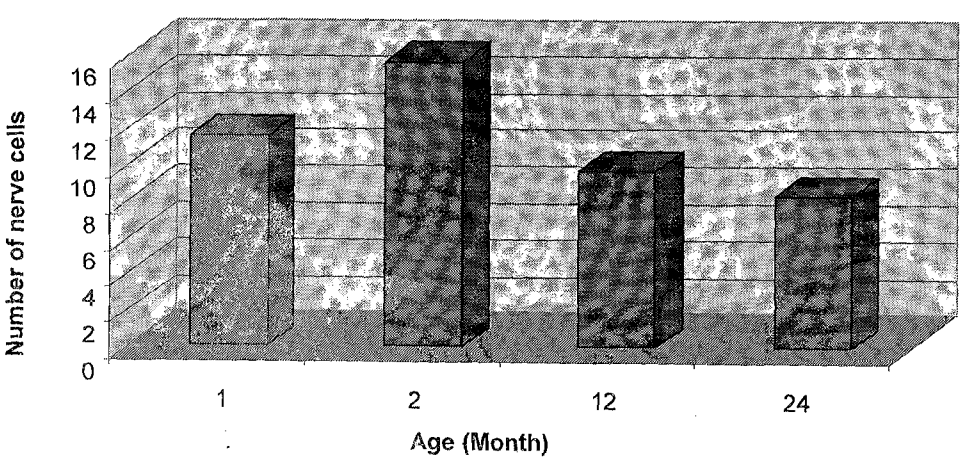


Fig. 2J: shows intramuscular myenteric plexus (MP) composed of unmyelinated nerve fibers. The smooth myofibers (SMF) of the muscularis externa are separated by wide intercellular spaces occupied by collagen fibers.

Graph (1): Showing the mean values of different measures taken in transverse sections of mice colon at different ages



Graph (2): Showing the mean number of nerve cells of Auerbach's Plexus/ certain field in mice colon in different age groups



they discharge their entire content of mucin into the lumen (Chang and Nadler, 1975). In the present study, goblet cells were apparently decreased in old aged mice. This finding could be due to extrusion of large number of goblet cells together with marked decrease in the formation of new ones. Since goblet cells were responsible for adding mucous to the dehydrated ingesta, the decreased goblet cells might lead to decreased mucous secretion rendering the mucosa prone to bleeding (Boley et al., 1977). Necrotic epithelial cells in the crypts of the old aged mice colon were observed in this study. Similar necrotic epithelial cells were detected in the human colon and were described as necrotic colonocytes (Barkla and Gibson, 1999).

In the present investigation, cellular infiltration was seen in the submucosa of the colon of old aged mice. In addition, dilated submucosal blood vessels were observed. Cellular infiltration might lead to disturbance of blood supply with subsequent hypoxic injury (Kasper, 1999).

In the present work, scanning electron microscopic examination revealed the presence of flat epithelial cells adjacent to the crypt openings and rounded epithelial ones in the intercryptal zones. It was observed that the intercryptal surface epithelium was more loosely attached to the basement membrane than the epithelium surrounding the crypts and that surface cells at the periphery of intercryptal territories are presumably older than those emerging from the crypt (Barkla and Gibson, 1999). These findings also support the recycling model of epithelial cell death in the surface compartment of the human colon (Barkla and Gibson, 1999; Shibahara et al., 1995). In the present study, the surface epithelium in old aged mice showed many small dark spots as well as areas of denuded epithelium. These dark spots were most likely empty goblet cells that had been collapsed (Barkla and Gibson, 1999). This explanation was confirmed by the apparent decrease in the mucous secretion on the luminal surface of the colon of old aged mice. The epithelial denuded areas most probably resulted from detachment of necrotic epithelial cells.

In the present study, transmission electron microscopic examination revealed that in the young group the intercellular space of the mucosal epithelium was dilated and filled by interdigitating membrane leaflets. In contrast there was a marked decrease in the microvillar system and in the intricate system in the epithelium of the colon of old age group. Finkel (1987) has previously reported that sodium and fluid absorption in the colon was higher in young than in old rats. Therefore, the present result may suggest that the observed reduction in the microvillar and intricate systems is one of the mechanisms underlying malabsorption in old age.

Examination of electron micrographs has shown that the cells in the lower one-third of the colonic crypts of the young mice included few apoptotic and necrotic cells. Many reports are available describing cellular differentiation in the colonic epithelium in the rat (Baba et al., 1999), the mouse (Menard et al., 1994) and in human (Altman, 1990). The presence of cells undergoing apoptosis and necrosis in the colonic epithelium was considered part of the normal process of cellular differentiation and renewal (Strater et al., 1995; Mandir et al., 2005). The present results have shown that the lower one-third of the colonic crypts of the young mice included only a few undifferentiated stem cells while that of the old mice contained numerous undifferentiated stem cells. This result is consistent with that of Xiao et al. (2001) who reported that aging is associated with increased proliferation and decreased apoptosis in the colonic mucosa of the rat, and that these changes may be responsible for the age-related rise in colorectal cancer.

The origin, location, morphology, function and identification of interstitial cells of Cajal in the myenteric plexus has been reported in detail (Radenkovic´ et al., 2005). These cells act as pacemakers of the gastric and intestinal musculature and were implicated in the modulation of enteric neurotransmission (Ëamborová et al., 2003; Radenkovic´ et al., 2005). The present work has demonstrated the presence of interstitial cells of Cajal in the

myenteric plexus of the colonic muscle coat in the young group whereas such cells were not identifiable in the old group. This may indicate a reduction in their number and (or) alteration in their ultrastructure. It has been indicated that changes in the number or in the ultrastructural characteristics of these cells may lead to various intestinal disorders (Long et al., 2004: Radenkovic´ et al., 2005).

The present study has demonstrated by statistical analyses that there was a significant decrease in the number of nerve cells in the myenteric plexus of two years old mice. Similar findings were observed in the old aged guinea pigs (Gabella, 1989; Wade,2002) and rats (El-Salhy et al., 1999; Phillips et al., 2003).Moreover, the number of degenerating neurons in the enteric ganglia was remarkably increased in old aged animals (Gabella, 1989; Phillips et al., 2003) and in the colon of old human subjects (Hanani et al., 2004).

The markedly reduced number of neurons in the myenteric plexus of old aged mice colon observed in this study, in aged rats(Phillips et al. ,2004) and in aging human colon (Gomes et al., 1997) seemed to affect the functions of the enteric nervous system and consequently, the potency of its varying actions on colonic functions might be also reduced.

Constipation is a common complain in the elderly (Schaefer and Cheskin, 1998).Slow colonic transit and increased rectal compliance were more common in old individuals who suffered from constipation than young adults (Merkel et al., 1993). Elderly patients might have diminished rectal sensitivity to dilatation and thus don't feel the urge to defecate (Meyer, 1991).

The present work thus suggests that the observed decrease in the number of mucosal goblet cells and microvillar and intricate systems, in the number of neurons and interstitial cells of Cajal in the myenteric plexus and in the muscle wall thickness, with an increase in deposition of collagen fibers in the mucosa and submucosa of the colon of old-aged mice probably contribute to colon dysfunction in elderly.

REFERENCES

Altman GG (1990): Renewal of the intestinal epithelium: new aspects as indicated by recent ultrastructural observations. J. Electron Microsc. Tech.16 (1): 2-14.

Altman DG (1994): Practical Statistics for Medical Research. Chapman and Hall, London, N.Y., Tokyo. 205-328.

Baba R, Tanaka R, Fujita M, Miyoshi M (1999): Cellular differentiation of absorptive cells in the neonatal rat colon: an electron microscopic study. Med. Electron Microscopy, 32(2):105-113.

Bancroft J D, Gamble M (2001): In Theory and Practice of Histological Techniques.5th edition, Churchill Livingstone Pub., Edinburgh and London.

Barkla DH, Gibson PR (1999): The fate of epithelial cells in the human large intestine. Pathology 31: 230-238

Boley SJ, Sammartano R, Adams A, Di Biasse A, Klienhaus S, Sprayregen S (1977): On the nature and etiology of vascular ectasias of the colon. Degenerative lesions of aging. Gastroenterology 72: 650-660.

Èamborová P, Hubka,P, Šulková I, and Hulin I (2003): The pacemaker activity of interstitial cells of Cajal and gastric electrical activity. Physiol. Res.52:275-284.

Camilleri M, Lee JS, Viramontes B, Bharucha AE,Tangalos EG (2000): Insights into the pathophysiology and mechanisms of constipation, irritable bowel syndrome and diverticulosis in older people. J. Am. Geriatr. Soc. 48: 1142-1150.

Chang WWL, Nadler NJ (1975): Renewal of the epithelium in the descending colon of the mouse in cell population kinetics of vacuolated columnar and mucous cells. Am. J. Anat., 144: 39 - 56.

Collure DW, Hameer HR (1996): Loss of ganglion cells and marked attenuation of bowel wall in cecal dilatation. J. Surg. Res. 60: 385-388.

Cormack DH (1987): "Ham Histology" 9th ed, J.B. Lipincott company, Philadelphia, London, Mexico, New York, St. Lois, Sao Paulo, Sydney. 513-515.

El-Salhy M, Sandstrom O, Holmlund F (1999): Age induced changes in the enteric nervous system in the mouse. Mech. Ageing Dev. 107: 93-103.

Finkel Y, Larsson L (1987): Morphometric and functional studies of proximal and distal colon in young and adult rats. J. Pediatr. Gastroenterol. Nutr. 6(3): 454-9.

Furness J, Costa M (1987): The enteric nervous system. London; Churchill living-stone. 65-69.

Gabella G (1989): Fall in the number of myenteric neurons in aging guinea pigs. Gastroenterology 96: 1487-1493.

Glauret AM (1986): Fixation, Dehydration and Embedding of Biological specimens. In Practical Methods in electron Microscopy. 1st edition. Amsterdam, New York, Oxford: North Holland publishing company, 111-1 48.

Gomes OA, De Souza RR and Liberti EA (1997): A preliminary investigation of the effects of aging on the nerve cell number in the myenteric ganglia of the human colon. Gerontology. 43: 210-217.

Hanani M, Fellig Y, Udassin R, Freund HR (2004): Age-related changes in the morphology of the myenteric plexus of the human colon. Auton Neurosci. 113: 71-78.

Kasper H (1999): Diseases of the large intestine in the aged. Constipation-diverticulosis-tumors-ischemia. Fortschr. Med. 108: 647-6 50.

Long QL, Fang DC, Shi HT, Luo YH (2004): Gastro-electric dysrhythm and lack of gastric interstitial cells of Cajal. World J. Gastroenterol. 10(8):1227-1230.

Mandir N, FitzGerald AJ, Goodlad RA (2005): Differences in the effects of age on intestinal proliferation, crypt fission and apoptosis on the small intestine and the colon of the rat. Int. J. Exp. Pathol.86 (2):125-130.

Meek GA (1970): Practical electron microscopy for biologists. 2nd dition. Chichester, New York, Brisdane, Toronto: John Willey and Sons, 413-470.

Menard D, Dagenais P, Calvert R (1994): Morphological changes and cellular proliferation in mouse colon during fetal and postnatal development. Anat. Rec.238(3):349-359.

Merkel I S, Locher J, Burgio K, Towers A, Wald A (1993): Physiologic and psychologic characteristics of an elderly population with chronic constipation. Am. J. Gastroenterol. 88: 1854-1859.

Meyer WB (1991): Motility of the large intestine: from irritable colon to constipation. Ther. Umsch; 48: 488-493.

Phillips RJ, Kieffer EJ, Powley TL (2003): Aging of the myenteric plexus; neuronal loss is specific to cholinergic neurons, Auton Neurosci. 31; 106: 38-69.

Phillips RJ, Kieffer EJ, Powley TL (2004): Loss of glia and neurons in the myenteric plexus of the aged Fischer 344 rat. Anat. Embryol. (Berl). 209(1):19-30.

Radenkovic´ G, Nikolic´ I and Todorovic´ V (2005): Interstitial cells of Cajal-pacemakers of the intestinal musculature. Medicine and Biology, 12(1):1-5.

Robinson DC, Uhler V, Herken R, Mayer F and Schurmann FW (1997): Methods for preparation for electron microscope. Pbl. Springer-Verlag. Berlin, New York , p 23 - 66 and 148-164.

Schaefer DC, Cheskin LJ (1998): Constipation in the elderly. Am. Fam. Physician. 58: 907-914.

Shibahara T, Sato N, Waguri S (1995): The fate of epithelial cells at the villous tips of the human small intestine. Arch. Histol. Cytol. 58: 205-219.

Strater J, Koretz K, Gunthert A R, Moller P (1995):In situ detection of enterocytic apoptosis in normal colonic mucosa and in familial adenomatous polyposis.Gut.37(6):819-825.

Watters JM, Redmond ML, Desai D, Mach RJ (1990): Effects of age and body composition on the metabolic responses to elective colon resection. Ann. Surg. 212(2): 213-220.

Wedel T, Roblick UJ, Gleiss J, Schiedeck TH, Bruch HP, Kuhnel W, Krammer HJ (1999): Organization of the enteric nervous system in the human colon demonstrated by whole mount immunohistochemistry with special references to the submucous plexus. Anat Anz. 181: 327-337.

Wade PR (2002): Aging and neural control of the GI tract: .I. Age-related changes in the enteric nervous system. Am. J. Physiol. Gastrointest. Liver Physiol. 283(3): G489-G495.

Wedel T, Spiegler J, Soellner S, Roblick UJ, Schiedeck TH, Bruch HP, Krammer H J (2002): Enteric nerves and interstitial cells of Cajal are altered in patients with slow-transit constipation and megacolon. Gastroenterology 123: 1459-1467.

Xiao ZQ, Moragoda L Jaszewski R, Hatfield JA, Fligiel SE, Majumdar AP (2001): Aging is associated with increased proliferation and decreased apoptosis in the colonic mucosa. Mech. Ageing Dev.122 (15):1849-1864.