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Morphological Evidence for a Cecocolonic Junction in Man and Functional Implications

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Abstract
The region above, below and in front of the ileocecal valve opening has been studied in man using both light and electron microscopy. A cecocolonic junction, comprising the colonic basal portion of the ileocecal valve, could be demonstrated in man, due to the specific anatomy of the inner portion of the circular muscle. This muscle was arranged in anastomosing cords, richly innervated and enveloped by elastic fibers. Its smooth muscle cells were characterized by extremely wide sarcoplasmic cisternae and cell-to-cell junctions, numerous caveolae and large amounts of glycogen. Interstitial cells were rarely found. This junction might be considered responsible for (1) ileal flow accommodation, (2) colonic active movements and (3) ileocecal valve closing and opening.

Introduction

Light- and electron-microscopic studies have shown cytoarchitectures peculiar to the human colonic muscle coat that have been related to the colon-specific motor activities [1–3]. The segments studied were obtained from the fundus of the cecum and from the ascending, transverse and descending colon. Conversely, no information is available on the morphological organization of the colonic region located around and in front of the ileocecal junction. This region has also received little attention in functional studies; nonetheless, physiological data seem to indicate that several important and specific motor activities take place there [4–12].

In the present study we focused our attention on the cytoarchitecture of the muscle coat of this portion of the human colon. According to the literature, the colonic ring we examined should correspond to the region where a cecocolonic sphincter is located. This sphincter, well developed in several animal species [13], has usually been considered to be absent or rudimentary in man [13–16], whereas a sphincteric region at the ileal base of the ileocecal valve has been ascertained for all animal species, man included [13, 14].

In this study, specific attention has been given to the inner portion of the circular muscle, since important morphological differences between the inner and outer portion of the colonic circular muscle layer characterize man [2] and other animal species [17–20]. The cytoarchitecture of the inner portion varies according to the colonic segments [2], suggesting marked differences between the right and left colon. Region-specific functional properties have been identified [21–23].

The aim of this work was to ascertain in the colonic region, which is assured to correspond to the cecocolonic junction, whether (1) structural characteristics of the circular muscle layer were present and (2) whether these peculiarities were similar to those found at other human colonic

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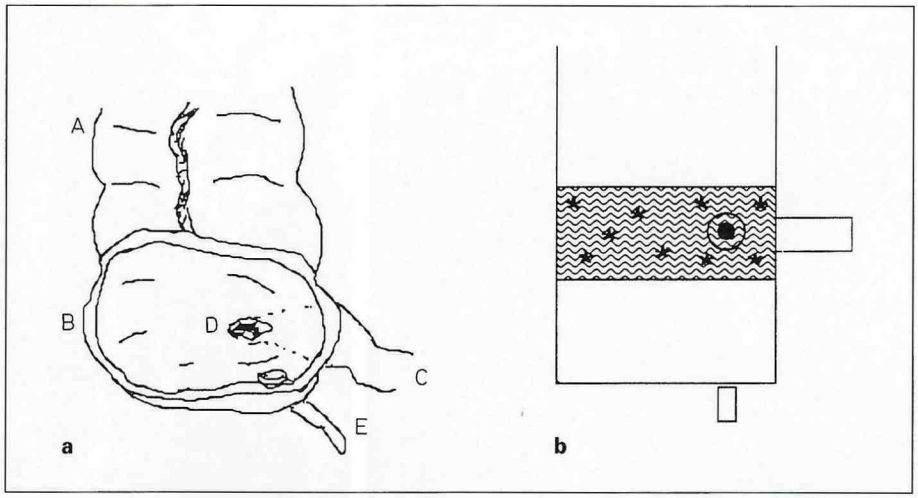


Fig. 1. **a** 'En face' view of the ileocecocolonic region. A = Ascending colon; B = cecum; C = terminal ileum; D = ileocecal valve opening; E = appendix. **b** Diagram showing the colonic area examined. Asterisks indicate where specimens were cut from. The hatched surface should approximately correspond to the extension of the cecocolonic junction.

levels or (3) were specific of this region. An organization of the circular muscle specific of this region might provide morphological evidence of a cecocolonic junction also in man. Furthermore, the nature of the structural peculiarities of this junction should provide an indication whether or not a sphincteric structure exists at this level and, consequently, of its role in man.

Material and Methods

Fragments of the cecocolonic junction were obtained from 5 patients (57–68 years old, mean age 60.16 years) undergoing right hemicolectomy for Dukes' A carcinoma of the proximal (right) transverse colon. The segments of both the terminal ileum and proximal colon had a normal appearance and were histologically free of tumor and inflammation. The patients had not taken any drugs affecting colon motility.

Immediately after surgery, the last 5–10 cm of the terminal ileum, the entire cecum and 10 cm of the ascending colon were separated from the remaining colon. Then, the cecum and the proximal part of the ascending colon were opened along the colonic anterior taenia in order to obtain an 'en face' view of the ileocecal valve (fig. 1a). Specimens of 1 cm^2 were cut all around the circumference of the colonic region, where the ileocecal valve opening is located. Some specimens were obtained from the colon adjacent to both the inferior and superior lips of the valve and some laterally to the valve, at various distances from its opening, and in front of it (fig. 1b). All specimens [except those used for the zinc iodide and osmium (ZIO) technique, see below] were distended with the submucosal surface down and pinned, in order to avoid spiralling and/or shortening of the muscle layers, on cork tablets provided with several holes, 0.3 cm in diameter. Preferably, areas showing a disarray of the muscle layers und unsuitable distension of their smooth muscle cells were excluded from both light- and electron-microscopic examination.

Light Microscopy

Some specimens were fixed by immersion in Bouin's solution (formaldehyde 40% m/v: 5 ml; picric acid solution 1.2%: 15 ml; glacial acetic acid: 1 ml). After alcoholic dehydration and toluene diaphanization, these specimens were embedded in paraffin. The sections, 5–7 μm thick, were stained with hematoxylin-eosin for general architecture viewing, with the periodic acid-Schiff reaction (PAS) for glycogen detection and paraldehyde-fuchsin solution (0.5% fuchsin, 1% hydrochloric acid, 1% paraldehyde) for elastic fiber staining. Immediately after resection, other specimens were immersed in a Champy-Maillet solution (ZIO) [20] to stain nerve tissue and interstitial cells of Cajal (ICC). These specimens could not be distended like others, since the metallic tools used to pin specimens on cork tablets interfere negatively with this osmic solution. All specimens were photographed under a Leitz Orthoplan light microscope.

Electron Microscopy

The distended and pinned specimens, as described above, were prefixed in a solution of 2% cacodylate-buffered glutaraldehyde, pH 7.4, and kept in this controlled distension for 8–10 h. Then, the pinned surfaces of each specimen were resected, and the mucosa was removed by sharp dissection. A thin layer of submucosa, however, remained attached. These segments were cut into strips of about 1 mm thickness and 2 mm length and rinsed in a buffered solution of saccharose. Then, they were postfixed with 1% phosphate-buffered OsO_4, pH 7.4, dehydrated with graded acetone, and embedded in Epon using flat moulds to obtain transverse or longitudinal sections. The semithin sections were stained with a solution of toluidine blue, and the area to be further cut for electron-microscopic examination was determined. These sections were also photographed under the light microscope. The ultrathin sections, cut on Porter-Blum MT1 or LKB Nova ultratomes using a sapphire knife, were stained with an alcoholic solution of uranyl acetate followed by a solution of concentrated bismuth subnitrate and examined under Siemens Elmiskop IA and 102 electron microscopes.

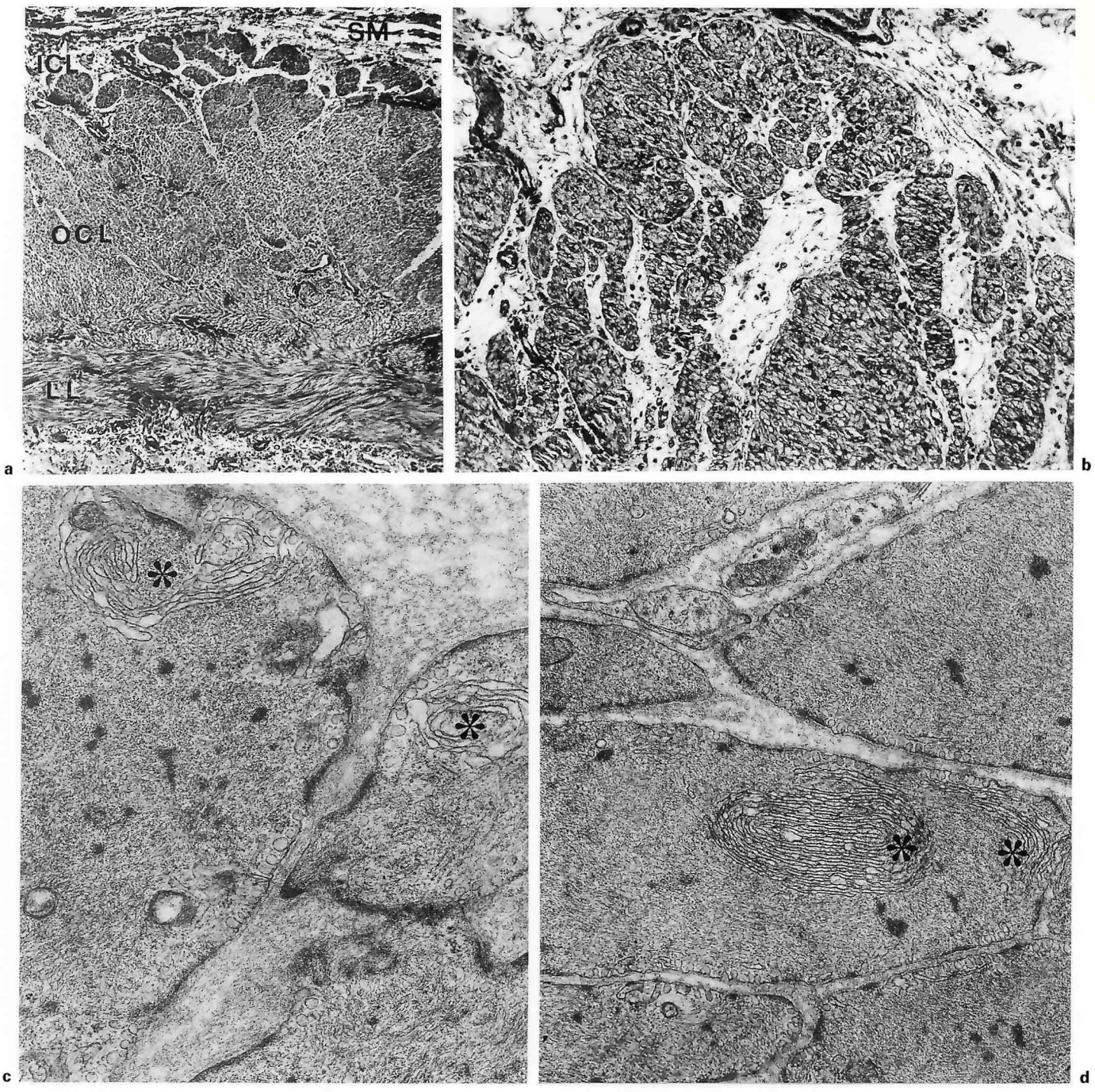
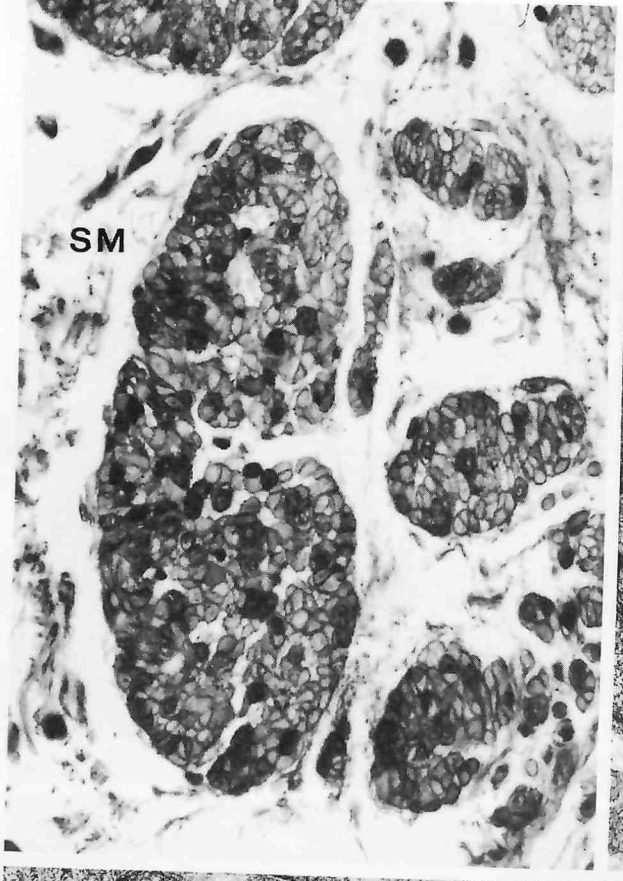


Fig. 2. Muscle coat of the cecocolonic junction. **a** LL = Longitudinal muscle layer; OCL = outer (main) portion of the circular muscle layer; ICL = inner portion of the circular muscle layer; SM = tela submucosa. Hematoxylin-eosin. ×80. **b** Inner portion of the circular muscle layer made up of muscle cords connected with each other and with the outer portion of the circular muscle layer. PAS. ×150. **c, d** Detail of organization and extension of SER cisternae (asterisks). **c** ×25,000. **d** ×20,000.

Fig. 3. **a** Glycogen content detected by the PAS reaction. Most of the smooth muscle cells of the inner circular muscle are deeply stained. SM = Tela submucosa. PAS. ×600. **b–e** The cytoplasmic areas containing glycogen particles (asterisks) and the cell-to-cell junctions are extremely wide. Numerous caveolae and long, flattened SER cisternae are distributed all along the cell-to-cell junctions. ×25,000.



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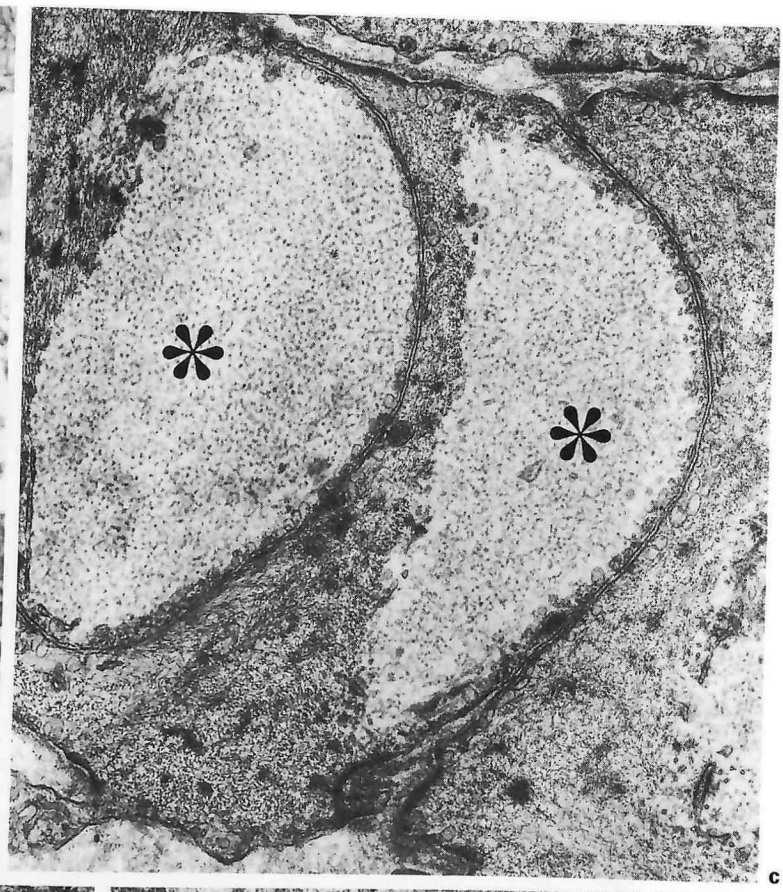

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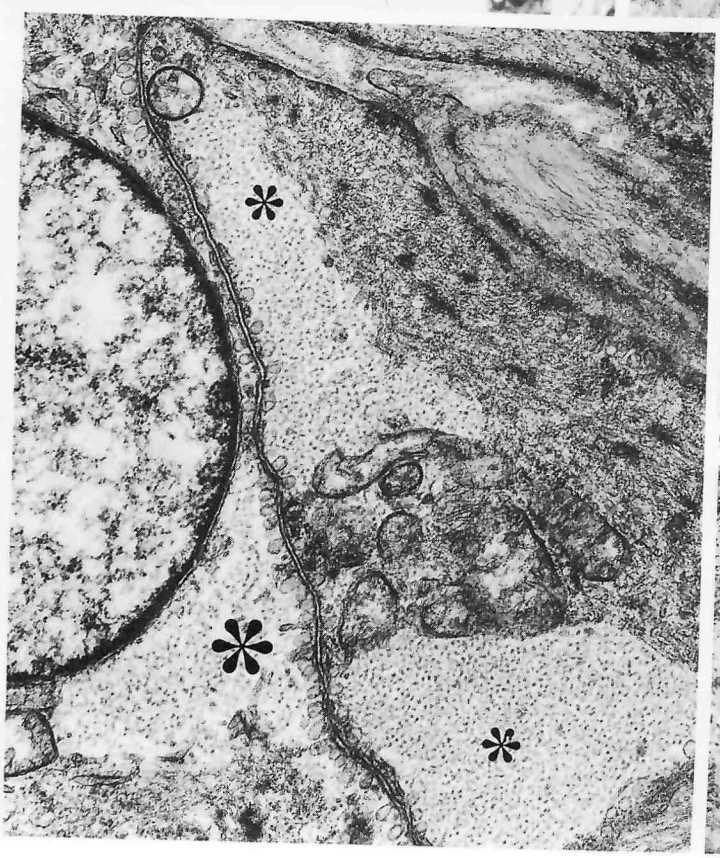

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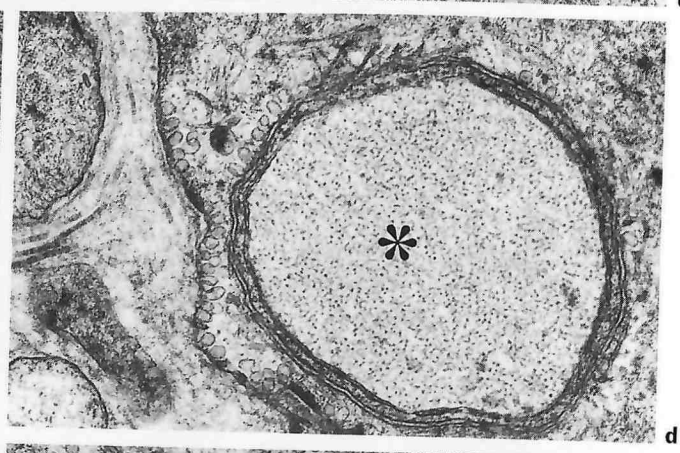

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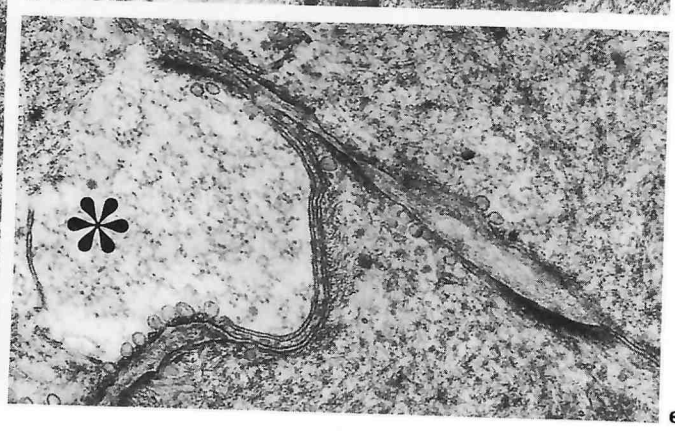

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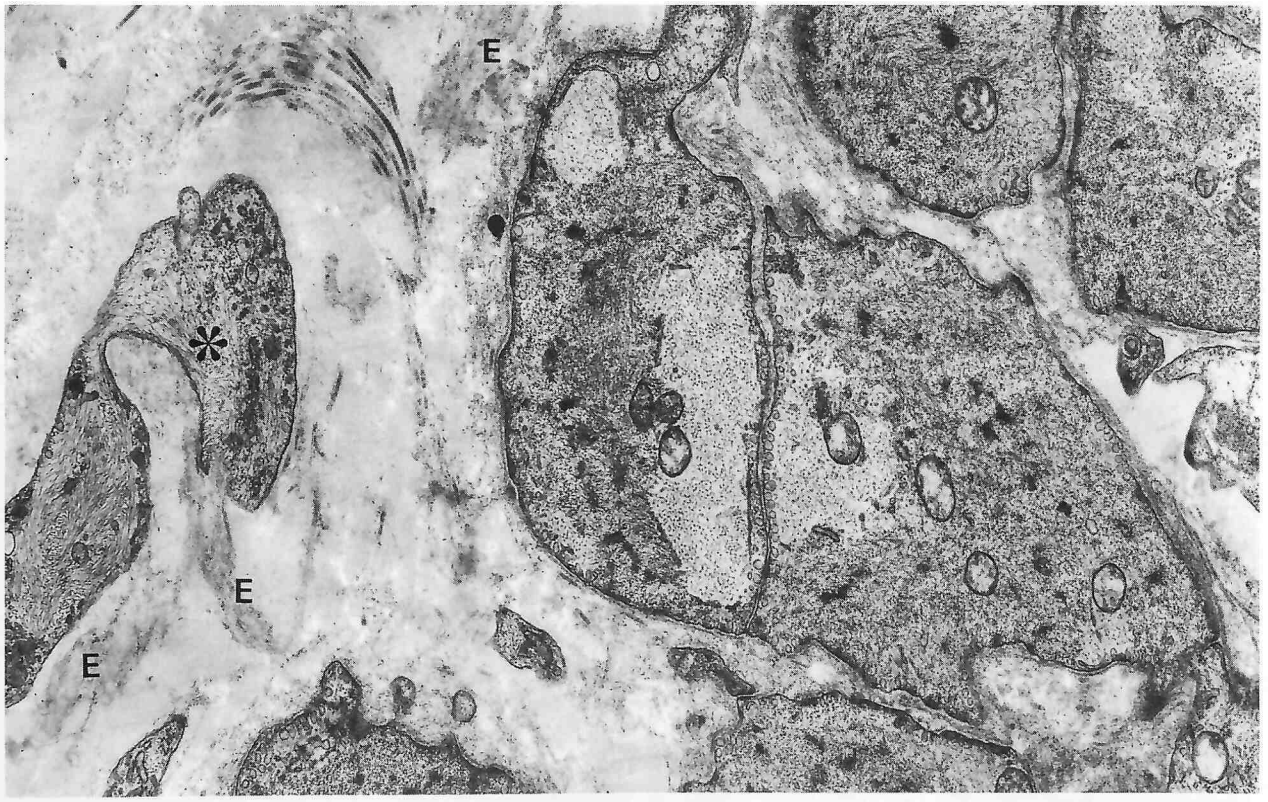

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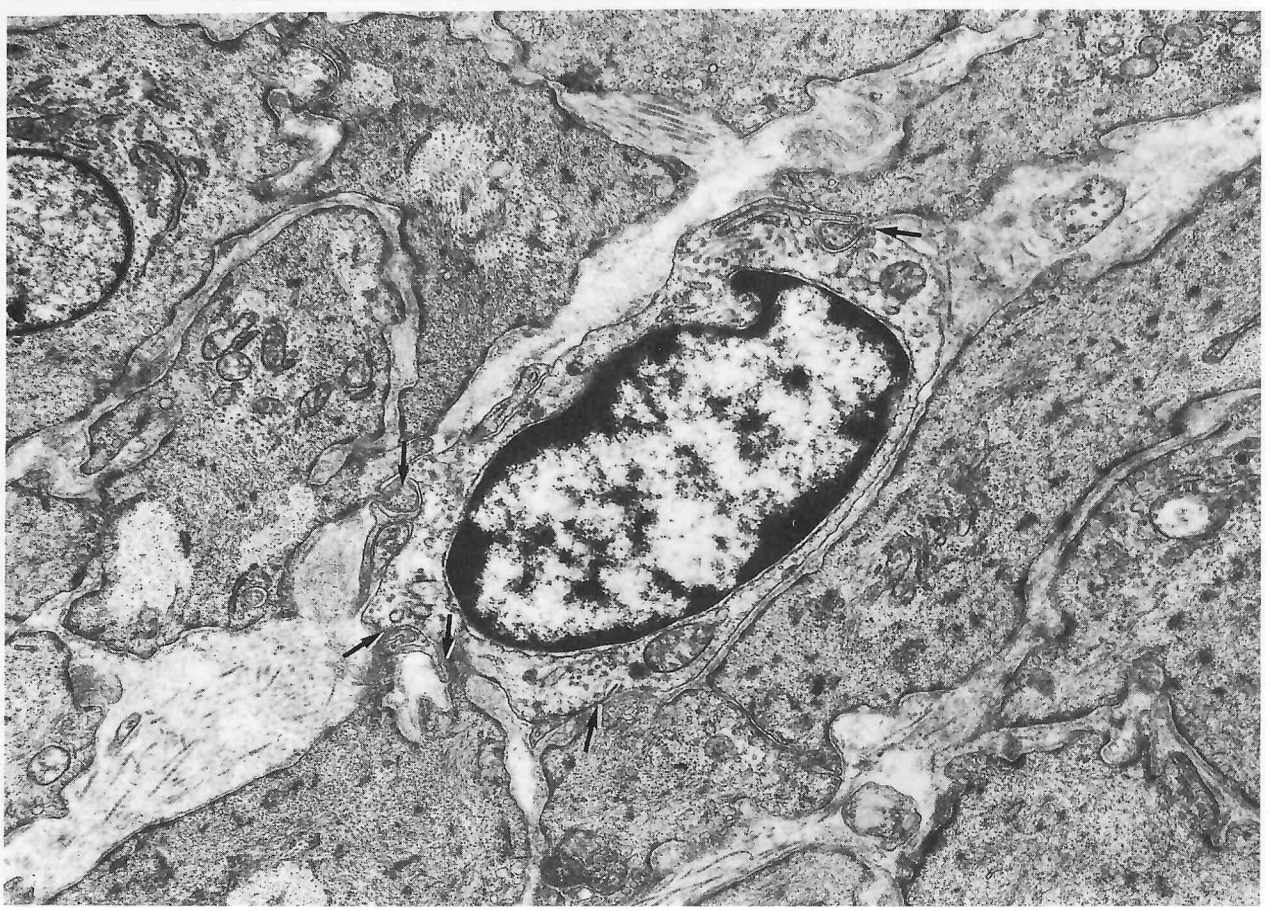

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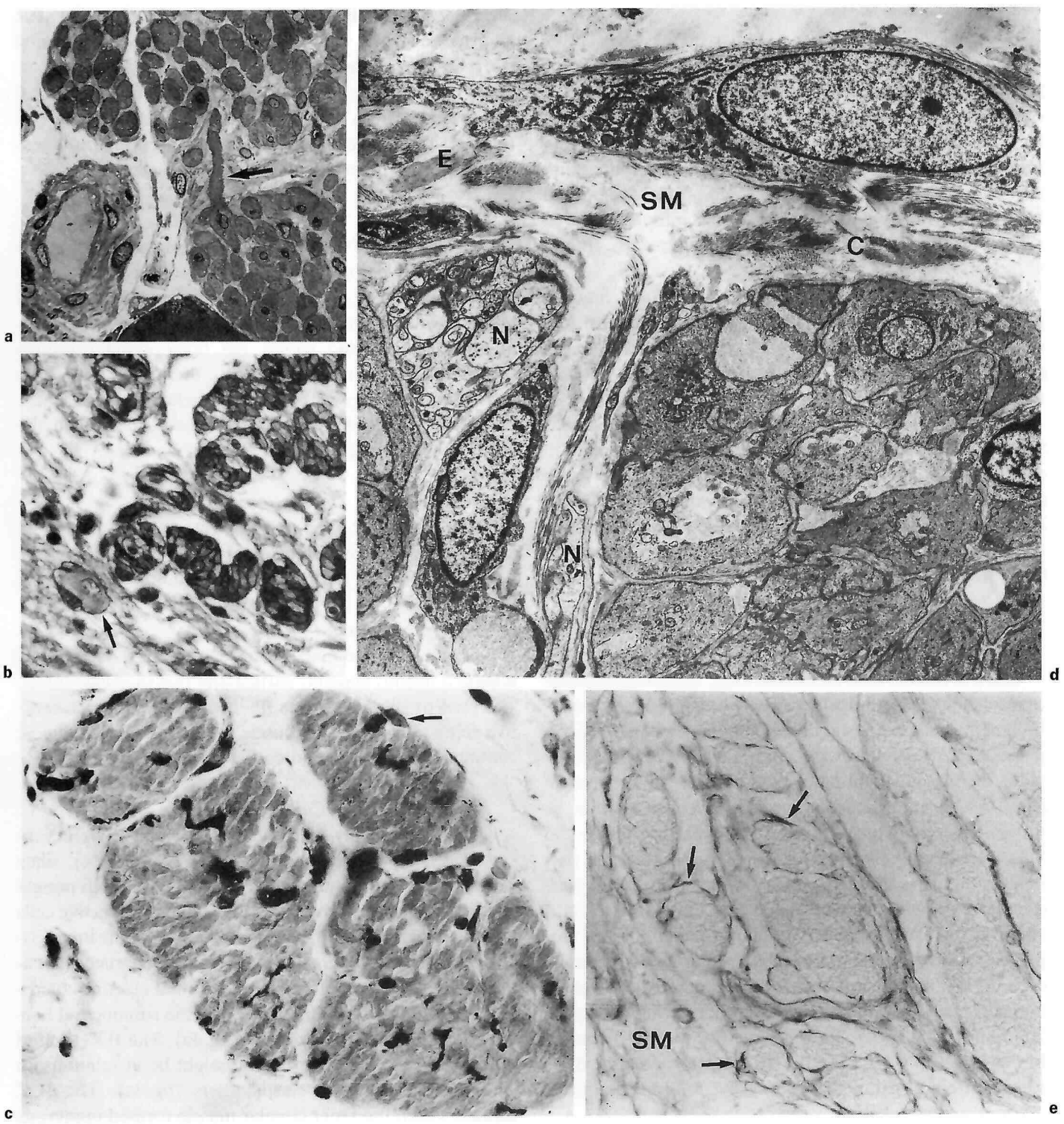

Fig. 5. **a** Inner circular muscle transversely sectioned. The arrow indicates a smooth muscle cell which extends from one muscle cord to the contiguous one. Semithin section, toluidine blue. ×450. **b** A ganglion of the plexus submucosus (arrow) near the submucosal border of the inner circular muscle. PAS. ×600. **c** Nerve fibers stained black with the Champy-Maillet (ZIO) technique, both close and within the muscle cords. The arrow indicates a presumptive gray-stained ICC. ×450. **d** Submucosal border of the inner portion of the circular muscle layer. SM = Tela submucosa; N = nerve fibers; E = elastic fibers; C = collagen fibers. ×5,000. **e** Elastic fibers (arrows) close and around the muscle cords. SM = Tela submucosa. Paraldehyde-fuchsin. ×500.

Results

Peculiar features have been observed in the colonic region, which is assumed to correspond to the presumptive cecocolonic junction in man (fig. 1b). The circular muscle layer in all specimens examined was about 1.2–1.8 mm thick and composed of two distinct portions, an inner and an outer circular muscle (fig. 2a), incompletely separated from each other by wide septa. These muscles differed markedly as for cell type population and connective framework.

The *outer circular muscle* was 0.9–1.3 mm thick. Smooth muscle cell cytology, nerve and blood vessel support and connective stroma were identical to those described in the other parts of the gut, especially the colon [1–3]. No ICC were observed within this muscle.

The *inner circular muscle* was 0.3–0.5 mm thick. Narrow, ramified connective tissue septa, in continuation with the tela submucosa and with the connective tissue separating the two circular muscles, divided it into anastomosing muscle cords (fig. 2b). A characteristic smooth muscel cell cytology, nerve and vascular support and connective stroma were observed. Moreover, ICC were also found.

Smooth Muscle Cells

Smooth muscle cells contained long, flattened cisternae of the sarcoplasmic reticulum (SER) which formed threads both at the cell periphery (fig. 2c) and deep within the cytoplasm (fig. 2d). Long rows of caveolae were distributed all along the plasma membrane. Most of these smooth muscle cells (fig. 3a) were particularly rich in glycogen content, as histochemically detected by the PAS reaction under the light microscope. Electron-microscopically, glycogen particles gathered in groups to form extremely wide and preferentially peripheral cytoplasmic areas (fig. 3b–e). As a consequence, myofilament distribution was limited to cytoplasmic regions smaller than in the other smooth muscle cells (fig. 3b, c). Smooth muscle cells contacted each other along several micrometer wide surfaces, with a gap of 20 nm only (fig. 3b–e). No gap junctions were recognized, but caveolae (fig. 3b, c) and SER cisternae (fig. 3b, d, e) were densely distributed all along the cell-to-cell contact surfaces. As could be frequently observed, a flattened SER cisterna, as wide as the contact surface, was located between the plasma membrane and glycogen particles. Single smooth muscle cells were longitudinally arranged and extended from one muscle cord to the contiguous one (fig. 5a). These smooth muscle cells showed the same features as other inner circular smooth muscle cells.

Innervation

Small ganglia of the plexus submucosus, containing 1–3 neuronal cells per section, could be found near the inner circular muscle (fig. 5b). The ZIO technique demonstrated numerous nerve fibers and nerve endings close to the submucosal surface and within the inner circular muscle (fig. 5c). Electron-microscopically, these nerve endings showed relationships with smooth muscle cells and a synaptic vesicle morphology similar to those observed in the outer circular muscle (fig. 2d, 5d).

Vessels

Both blood and lymphatic vessels of small or medium caliber were very frequently found in the vicinity of the submucosal border of the inner circular muscle (fig. 2b, 5b).

Connective Stroma

All along the submucosal surface of the inner circular muscle a fibrous lamella, particularly rich in elastic fibers, was present (fig. 5d). These fibers penetrated the connective septa delimiting the muscle cords, forming an almost continuous sheath around each muscle cord (fig. 5e).

Interstitial Cells of Cajal

Cells stained gray with the ZIO technique were very rare and not positively identifiable as ICC (fig. 5c), since close contacts with both nerve endings and smooth muscle cells were never observed and gray-stained connective cells were also present at the submucosal border of the inner circular muscle. However, some cells with ultrastructural features characteristic of ICC were identified electron-microscopically both within (fig. 4b) and at the submucosal border of the inner circular muscle (fig. 4a). The ICC located outside the inner circular muscle might be in relationship with nerve fibers and elastic fibers (fig. 4a). The ICC located within the inner circular muscle formed numerous cell-to-cell junctions with the contiguous smooth muscle cells (fig. 4b).

Fig. 4. ICC. **a** An ICC (asterisk) located at the submucosal border of the inner circular muscle. E = Elastic fibers. ×15,000. **b** An ICC located within the inner circular muscle. Arrows indicate the cell-to-cell junctions between the ICC and the contiguous smooth muscle cells. ×15,000.

Discussion

The muscular wall of the circumferential colonic segment comprehensive of the ileocecal valve opening, the adjacent cecum and ascending colon, i.e. the presumptive cecocolonic junction, has been examined under both the light and electron microscope. A characteristic organization of the circular muscle was found in all segments examined, suggesting that a cecocolonic junction might be microscopically detected also in man. Since a similar organization was found in all specimens examined, the colonic basal portion of the ileocecal junction has to be included in this region. This thick, peculiarly organized circular muscle layer might indicate that a cecocolonic sphincter, such as in other animal species [13–16], exists also in man. The functional significance of the structural features of this muscle needs to be discussed in order to clarify (1) whether they characterize a sphincteric muscle or not and (2) its function in man.

Both under the light and electron microscope, the circular muscle of this region appeared clearly subdivided into two differently organized portions. While the outer part of the circular muscle did not differ from that of the other parts of the colon, the inner one differs with respect to smooth muscle cells, connective tissue stroma, nerve and vascular support and ICC. This inner circular muscle was arranged in anastomosing muscle cords, connected with each other and with the outer part of the circular muscle. The extension of the SER cisternae and the cell-to-cell junctions of these inner smooth muscle cells were enormous and caveolae and glycogen particles extremely numerous. The latter often occupied cytoplasmic areas so large that only a reduced space was left for myofilaments.

Typical of the cecocolonic inner smooth muscle cells was, therefore, their expanded Ca^{2+} storage/release apparatus (SER) and energetic material (glycogen) compared to the contractile apparatus (myofilaments), and the richness and extension of their cell-to-cell junctions. The latter data might conform to some functional requirements [6, 24], such as the ability to act in synchrony and propagate contraction in every direction.

Similar structural characteristics, even though present in a lesser number of cells and less pronounced in each cell, had already been found in the smooth muscle cells located all along the human colon at the submucosal border of the circular muscle layer [1, 2]. These cells, due to their cytology [25] and location [1, 2], were considered as pacemaker cells, and present data seem to indicate the presence of a pacemaker tissue also at the level of the cecocolonic junction. However, no functional data are available which indicate the presence or a need for a pacemaker tissue at the cecocolonic region. ICC, another type of presumptive pacemaker cells of the gut, were, as in other parts of the human colon, only rarely found at the cecocolonic junction. The colonic ICC contribution in slow wave origin seems, therefore, less important in humans than in other mammals [17, 22, 23, 26–28], where these cells are the sole cell type present at the level of the pacemaker area of the circular muscle layer.

Both the presence of a muscular pacemaker tissue and the absence of ICC give no indication about sphincteric properties of the cecocolonic circular muscle. On the other hand, the particularly rich innervation found at the cecocolonic junction, where nerve fibers are located also within the muscle cords, and the presence of a well-developed, and 'specific', inner circular muscle might account for a sphincteric function of this region.

In an attempt at a morphofunctional correlation, we also have to consider that the cecocolonic junction in man might be responsible for some other properties peculiar to the ileocecal region. In this sense, the richness in elastic fibers found at the submucosal border and all around the inner muscle cords might be responsible for passively accommodating the lumen diameter of the cecum, and the similarities between the structural characteristics of the 'basal colonic' portion of the valve and those of all other parts of the cecocolonic junction might indicate that some motile properties of the ileocecal valve, such as opening and closing, might be due to its 'basal colonic' inner circular muscle, rather than to its 'basal ileal' circular muscle only.

In conclusion, the unique features found in the inner muscle collar surrounding the cecum at the junction of the cecum and the colon permit to detect a specialized region, the cecocolonic junction, whose functional implications in the motility of this part of the gut should not be underestimated. Up to date, structural peculiarities characterizing a sphincteric region have not yet been defined, the knowledge of the physiological behavior of such a region is scarce and present data are not sufficient to support any interpretation. However, in a purely speculative manner, we can suggest that this specialized inner circular muscle might be involved (1) in the active movements of the cecum, (2) in ileal flow accommodation and (3) ileocecal valve closing and opening.

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References

1 Faussone-Pellegrini MS, Cortesini C: Ultrastructural peculiarities of the inner portion of the circular layer of the colon. I. Research in the human. Acta Anat 1984;120:185–189.

2 Faussone-Pellegrini MS, Cortesini C, Pantalone D: Neuromuscular structures specific to the submucosal border of the human colonic circular muscle layer. Can J Physiol Pharmacol 1990;68:1437–1446.

3 Faussone-Pellegrini MS, Pantalone D, Cortesini C: Smooth muscle cells, interstitial cells of Cajal and myenteric plexus interrelationships in the human colon. Acta Anat 1990;139: 31–44.

4 Cohen S, Harris LD, Levitan R: Manometric characteristics of the human ileocecal junctional zone. Gastroenterology 1968;54:72–75.

5 Corazziari E, Barberani F, Tosoni M: Motor activity of the ileocecal valve (ICV) in man (abstract). J Gastrointest Motil 1989;1:48.

6 Huizinga JD, Daniel EE: Motor function of the colon; in Phillips S, Pemberton JH, Shorter RG (eds): The Large Intestine: Physiology, Pathophysiology and Disease. Mayo Foundation. New York, Raven Press, 1991, chapt 5, pp 93–114.

7 Huizinga JD, Stern HS, Diamant NE, El-Sharkawy TE: Electrophysiological control of motility in the human colon. Gastroenterology 1985;88:500–511.

8 Phillips SF, Quigley EMM, Kumar D, Kamath PS: Motility of the ileocolonic junction. Gut 1988;29:390–406.

9 Quigley EMM, Phillips SF, Cranley B, Taylor BM, Dent J: Tone of canine ileocolonic junction: Topography and response to phasic contractions. Am J Physiol 1985;249:G350–G357.

10 Sarna SK: Physiology and pathophysiology of colonic motor activity (part one of two). Dig Dis Sci 1991;36:827–862.

11 Sarna SK, Prasad KR, Lang IM: Giant migrating contractions of the canine colon. Am J Physiol 1988;254:G595–G601.

12 Torsoli A, Ramorino ML, Ammaturo MV, Capurso L, Paoluzzi P, Anzini F: Mass movements and intracolonic pressures. Am J Dig Dis 1971;16:693–696.

13 Pelckmans PA: Study on the Sphincteric Nature of the Ileocecal Junction: A Morphological and Pharmacological Approach; thesis University of Antwerp, 1988.

14 Didio LTA, Anderson MC: The 'Sphincters' of the Digestive System. Baltimore, Williams and Wilkins, 1968.

15 Gazet JC: The surgical significance of the ileocaecal junction. Ann R Coll Surg Engl 1968; 43:19–38.

16 Rutherford AH: The frenula valvulae coli. J Anat 1926;60:411–415.

17 Berezin I, Huizinga JD, Daniel EE: Interstitial cells of Cajal in the canine colon: A special communication network at the inner border of the circular muscle. J Comp Neurol 1988;273: 42–51.

18 Faussone-Pellegrini MS: Le caratteristiche ultrastrutturali della tunica muscolare dell'ileo e del colon del ratto. A. Porzione interna dello strato circolare. Arch Ital Anat Embriol 1983; 88:25–40.

19 Faussone-Pellegrini MS: Ultrastructural peculiarities of the inner portion of the circular layer of the colon. II. Research on mouse. Acta Anat 1985;122:187–192.

20 Stach W: Der Plexus entericus extremus des Dickdarmes und seine Beziehungen zu den interstitiellen Zellen (Cajal). Z Mikrosk Anat Forsch 1972;85:245–272.

21 Smith TK, Reed JB, Sanders KM: Origin and propagation of electrical slow waves in circular muscle of canine colon. Am J Physiol 1987; 252:C215–C224.

22 Ward SM, Burke EP, Sanders KM: Use of rhodamine-123 to label and lesion interstitial cells of Cajal in canine colonic circular muscle. Anat Embryol 1990;182:215–224.

23 Ward SM, Keller RG, Sanders KM: Structure and organization of electrical activity of canine distal colon. Am J Physiol 1991;260: G724–G735.

24 Huizinga JD, Waterfall WE: Electrical correlate of circumferential contractions in human colonic circular muscle. Gut 1988;29:10–16.

25 Rizzo M, Faussone-Pellegrini MS, Riccardi-Arbi R, Ponchietti R: Ultrastructure of the urinary tract muscle coat in man: Calices, renal pelvis, pelvi-ureteric junction and ureter. Eur Urol 1981;524:171–177.

26 Barajas-Lopez C, Berezin I, Daniel EE, Huizinga JD: Pacemaker activity recorded in interstitial cells of Cajal of the gastrointestinal tract. Am J Physiol 1989;257:C830–C833.

27 Du C, Conklin JL: Origin of slow waves in the isolated proximal colon of the cat. J Auton Nerv System 1989;28:167–178.

28 Langton P, Ward SM, Carl A, Norell MA, Sanders KM: Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon. Proc Natl Acad Sci USA 1989;86:7280–7284.