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New Insights into the Neuromuscular Anatomy of the Ileocecal Valve

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ABSTRACT

The neuroanatomy of the ileocecal valve (ICV) is poorly understood. A better understanding of this important functional component of the gastrointestinal tract would enable surgeons to reconstruct an effective valve following surgical resection of the ICV. ICVs were examined in young pigs (N = 5) using frontal and transverse paraffin embedded and frozen sections. Hematoxylin+Eosin (H+E) staining, acetylcholinesterase (AchE), and NADPH-diaphorase (NADPH-d) histochemistry and protein gene product 9.5 (PGP 9.5) and C-kit immunohistochemistry were performed. The H+E staining revealed that the ICV consists of three muscle layers: an external circular muscle layer continuous with that of the ileal circular muscle layer, an inner circular muscle layer continuous with that of the cecal circular muscle layer, and a single longitudinal muscle layer, which appears to be secondary to a fusion of the ileal and cecal longitudinal muscle layers. The AchE, NADPH-d, and PGP 9.5 staining revealed two distinct coaxial myenteric plexuses, together with superficial and deep submucosal plexuses. The C-kit immunostaining showed a continuous myenteric ICC network within the ICV. The structure of the neuromuscular components within the ICV suggests that the valve is a result of a simple intussusception of the terminal ileum into the cecum. This knowledge may help surgeons in their future attempts at reconstructing more anatomically and functionally suitable ICVs following surgical resection of native ICVs. Anat Rec, 000:000–000, 2008. © 2008 Wiley-Liss, Inc.

Key words: enteric nervous system; ileocecal valve replacement; ileocecal motility

The clinical importance of the ileocecal valve (ICV) resides in its loss. It has been shown by Quigley and Thompson (1994) and Bakkevold (2000), when it does not function properly, the fecal reflux increases the risk of the bacterial colonization of the terminal ileum resulting in bacterial overgrowth. The loss of control of the ileocecal antegrade flow reduces transit time and decreases absorption.

Several different surgical attempts have been made to replace the ICV. The surgical design of the created valve has been based on the actual concept of the ICV. The first macroscopic anatomical reports described the ICV as a mucosal fold into the lumen of the cecum and it was considered a passive valve structure providing reflux control (Kumar and Philips, 1987). After recognizing its importance in Crohn's disease, the surgical design aimed to create passive hydrostatic reflux control

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(Bakkevold, 2000). Mucosal valves or full thickness intussusception-like small bowel valves were created. Ricotta et al. (1981) published an "inside-out" ileum valve, Zurita et al. (2004) slipped the free end of the terminal ileum into the cecum for ileocolic anastomosis.

The concept of an active sphincter emerged after studies examining the microscopic anatomy described a thickened musculature. Quigley et al. (1985) also detected a high-pressure zone at the ileocecal junction, and the control of the antegrade flow of the sphincter became evident. It was consistent with the clinical finding of Willmore (1972) who recognized the risks of the surgical resection of the sphincter in short bowel syndrome.

Ecker et al. (1994) made attempts to create an intestinal neosphincter by ultrasonic tissue fragmentation.

In our previous study (Cserni et al., 2005), with the sphincter concept in mind, we were able to increase the passive antegrade hydrostatic efficacy of the surgically designed intussusception-like small bowel valves up to that of the physiological value of the ICV.

Manometric studies by Shafik et al. (2002) further demonstrated the inhibitory and excitatory reflexes of the ileocecal junction. The functional integrity and coordinated motility pattern of the terminal ileum-ICV/sphincter-cecum have been highlighted in a review of Malbert (2005). However, the anatomical basis has not been studied in the same detail as that of, for example, the gastroduodenal junction, where the anatomic isolation of the circular muscle fibers (Cai and Gabella, 1984) and gap in the myenteric interstitial cells of Cajal (ICC) network (Wang et al., 2005) has been found to be involved in uncoordinated gastroduodenal motility.

The information available about the enteric nervous system (ENS) in the ICV is sparse. A higher density of neurons has been reported in the ICV by Altdorfer et al. (1996) and Kaur et al. (2005), but detailed anatomical descriptions with primary reference to the structure of the ENS within the ICV has not been found in the literature to date. No previous study has focused on the presence of the ICC network in the ICV.

The lack of knowledge of the detailed neuroanatomy of the ileocecal junction turned our attention to studying the ENS in the ICV. The arrangement of the ganglion cells and the interstitial Cajal cell network was studied using histological techniques to enable surgeons reconstructing anatomically and functionally more suitable valve following surgical resection of the ICV.

MATERIAL AND METHODS

Specimens were taken from young piglets (N = 5). The animals were provided by the Institute of Experimental Clinical Research, Skejby Sygehus, University of Aarhus, Denmark. The study was approved by the Danish authorities of animal protection, permission number 200601-068. All of the five piglets selected for the study were 12-week old and weighed between 35 and 37.5 kg.

After killing the animals, the entire gastrointestinal tract was subsequently removed, washed in PBS, and subsequently immersed into 4% paraformaldehyde (PFA) solution for 4 hr at room temperature, and stored in PBS at 4°C and was used within 3–4 weeks.

The ileocecal junctions were cut and used for this study. Frontal and transverse sections of paraffin-embedded specimens were stained with conventional Hematoxylin–Eosin staining, and protein gene product 9.5 (PGP 9.5) and C-kit fluorescent immunohistochemistry. Frozen sections were used for acetylcholinesterase (AchE), NADPH-diaphorase (NADPH-d) histochemistry.

Thickness of the muscle layers was measured on photographs of frontal sections of frozen specimens at the base, middle, and at the free end of the valve using Image J software. Significance was calculated using one-way ANOVA test.

Acetylcholinesterase Histochemistry

The staining was carried out using the method of Karnovsky and Roots (1965) as modified by Kiernan (1990). The slides were placed in 10 mL of an incubating solution (65 mM sodium acetate buffer, pH 6.0, 1.7 mM acetylthiocholine iodide, 5 mM sodium citrate, 3 mM cupric sulfate, and 0.5 mM potassium ferricyanide) for 100 min at 37°C. Slides were then rinsed twice in 0.1 M Tris buffer (pH 7.6) and mounted using Glycergel mounting medium (Dakocytomation).

NADPH-d Histochemistry

For NADPH-d histochemistry, slides were incubated in 1 mg/mL β-NADPH (Sigma), 0.25 mg/mL nitro blue tetrazolium, and 0.3% Triton-X in 0.05 mol/L Tris-HCL buffer (pH 7.6) at 37°C for 2 hr, and then mounted using Glycergel mounting medium (Dakocytomation).

PGP 9.5 and C-Kit Fluorescent Immunohistochemistry

The slides were placed in citrate buffer (pH 6) and microwaved at 650 W for 7 min for antigen heat unmasking. They were then cooled down on ice for 45 min and washed in phosphate buffer 2 × 5 min. The samples were blocked with 10% goat serum (Dakocytomation) for 30 min at room temperature. After shaking off the blocking serum, the samples were incubated with rabbit anti-human PGP 9.5 antibody (Dakocytomation), diluted in antibody dilutant (1:500) or rabbit anti-human C-kit (Dakocytomation), diluted in antibody dilutant (1:50) overnight at 4°C. Specimens were washed in phosphate buffer 2 × 5 min and subsequently incubated with "Texas Red" fluorescent conjugated goat anti-rabbit antibody (Eugene, OR) diluted in 10% goat serum (1:100) at room temperature for 30 min. The slides were mounted with Fluorescein mounting medium by Dakocytomation. A Leica DC 300F fluorescent light microscope with an attached digital camera was used to visualize results.

RESULTS

Macroscopic Appearance of the Ileocecal Valves

The ileum opened into the large intestine, in all cases, at the level of the cecocolic junction in the form of a conical papilla. This is identical to previous observations by Prado and DiDio (2000).

Hematoxylin–Eosin Staining

The transverse and frontal sections revealed that the ileocecal sphincter consists of three distinct muscle layers: an external circular muscle layer continuous

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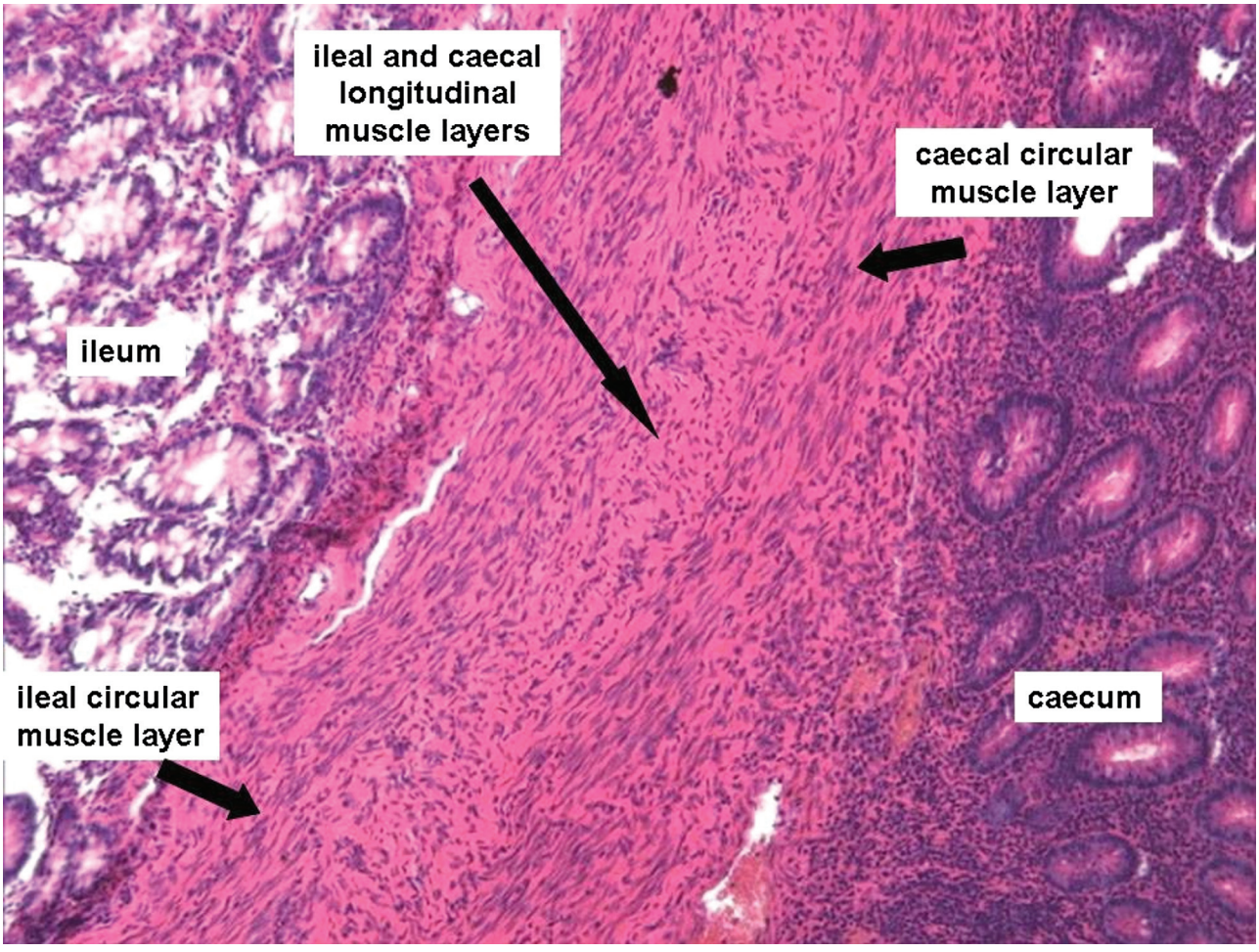

Fig. 1. Transverse section of a piglet ICV with Hematoxylin–Eosin staining (×40 magnification): There are two concentric circular muscle layers separated with the fused longitudinal muscle fibers.

with that of the cecal circular muscle layer, an inner circular muscle layer continuous with that of the ileal circular muscle layer. The two circular layers are connected at the free end of the valve.

The junction of the ileal and cecal circular muscle fibers appears as an annular sphincter described by DiDio (1968).

The single longitudinal muscle layer appears to be secondary to a fusion of the ileal and cecal longitudinal muscle layers. (Figs. 1 and 2.) F1 F2

There was minimal inter animal difference in the size of muscle layers (Table 1). The values of SD and SE were found very low. The ileal muscle layers seemed to be slightly thicker in all animals compared with the cecal circular muscle layers. The thickness of the ileal muscle layers was constant, the cecal circular muscle layer slightly increased, but the fused longitudinal layer significantly decreased in thickness along the ICV. T1

Acetylcholinesterase and NADPH-d Histochemistry, PGP 9.5 Immunohistochemistry

AchE and NADPH-d histochemistry and the PGP 9.5 immunohistochemistry showed two definite distinct coaxial origins of the ENS, separated by the muscle layers at the base and body of the ICV (Fig. 3). F3

The external ENS was seen to originate from the cecum and consists of superficial submucosal, deep submucosal, and myenteric plexuses as in the cecum. The internal ENS originates from the ileum and its structure resembles that of the ileum (Fig. 4). The PGP 9.5 immunostaining visualized the best direct connection between the ileal and cecal ENS at the free end of the valve. F4

C-Kit Immunohistochemistry

The C-kit immunostaining demonstrated the presence of myenteric interstitial cells of Cajal's in the ileal and cecal plexuses and did not reveal any break in continuity between the ileal and cecal ICC network (Fig. 4).

DISCUSSION

The ileocecal junction resembles the gastroduodenal junction, because in both cases adjacent organs of the gastrointestinal tract are joined but both have different functions in digestion. The coordination of gastrointestinal motility is different in these two junctions. Several

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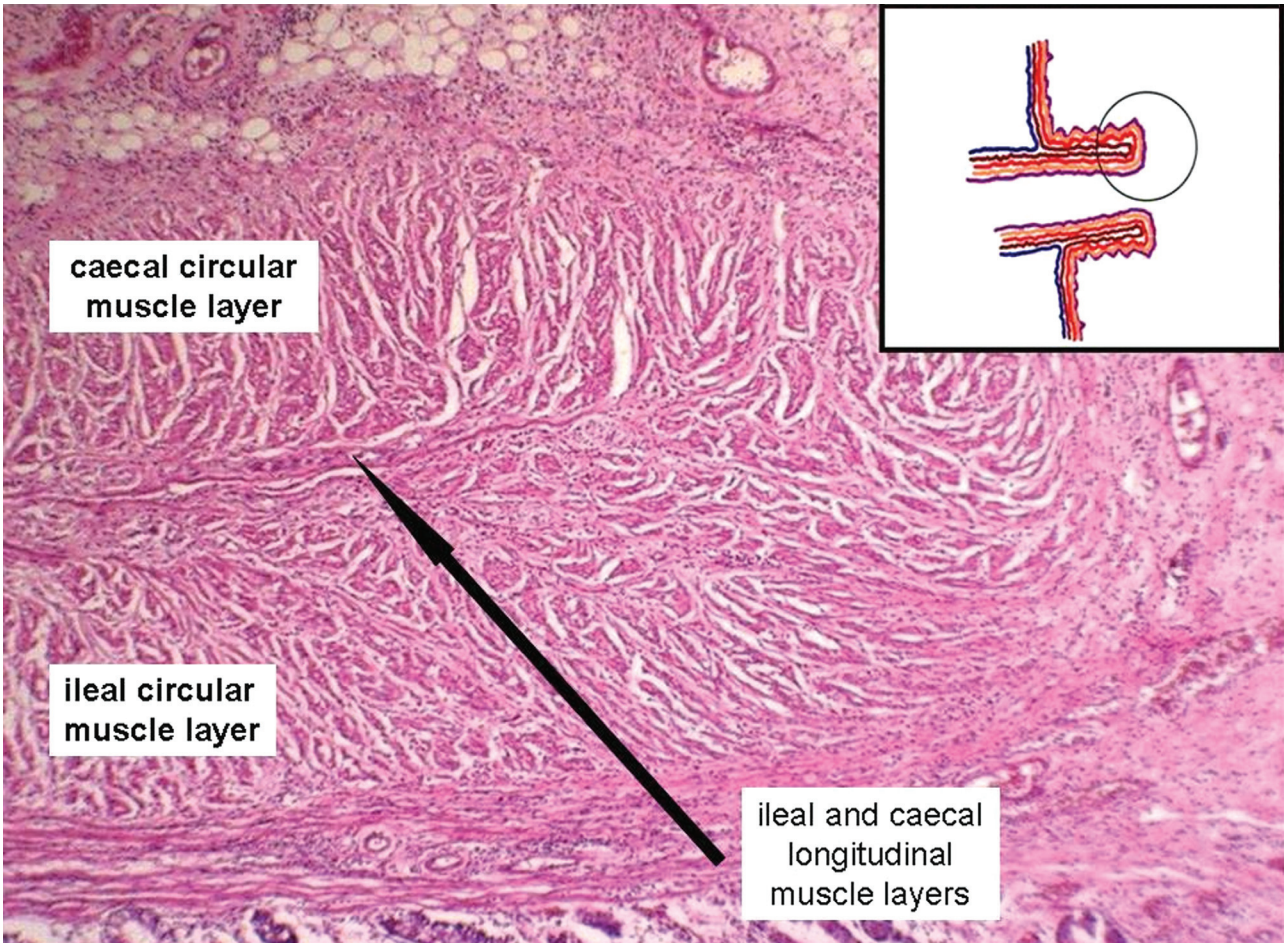

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Fig. 2. Frontal section of piglet ICV with Hematoxylin–Eosin staining (×40 magnification): The longitudinal muscle layers are separate from the circular muscle layers along the ICV but not at the free end of the valve.

TABLE 1. Thickness of the muscle layers (in mm) measured on frontal section of frozen specimens stained with H&E

	Thickness of lleal origin circular muscle layer within the value (mm)			Thickness of caecal origin circular muscle layer within the value (mm)			Thickness of fused longitudinal muscle layer within the value (mm)		
	Base	Middle	Free end	Base	Middle	Free end	Base	Middle	Free end
Mean	0.321	0.327	0.309	0.272	0.283	0.291	0.076	0.071	0.055
SD	0.016	0.012	0.013	0.007	0.011	0.012	0.008	0.009	0.011
									$P = 0.007$ vs.base
Significant differences						$P = 0.039$ vs.base			$P = 0.035$ vs.middle

Significance was calculated using one-way ANOVA test

authors, Quigley et al. (1985), Dinning et al. (1999), Hipper and Ehrlein (2001), Malbert (2005) found the motility of the ileocolonic junction mostly synchronized, in pigs 70% of ileal contractions are coordinated with colonic contractions. In contrast, according to Cai and Gabella (1984), Lammers et al. (1998), and Wang et al. (2005), muscular contractions are not normally propagated from the pyloric end of the stomach to the duodenum.

The anatomical background of the uncoordinated gastroduodenal motility is well studied, but information about the ileocecal junction was sparse. In our study, we have found that the muscular anatomy of the ICV does not resemble other gastrointestinal sphincters (pyloric, internal anal sphincter), in which homogenous thickening of the circular muscle layer has been observed. The ICV consists of two circular muscle layers with constant

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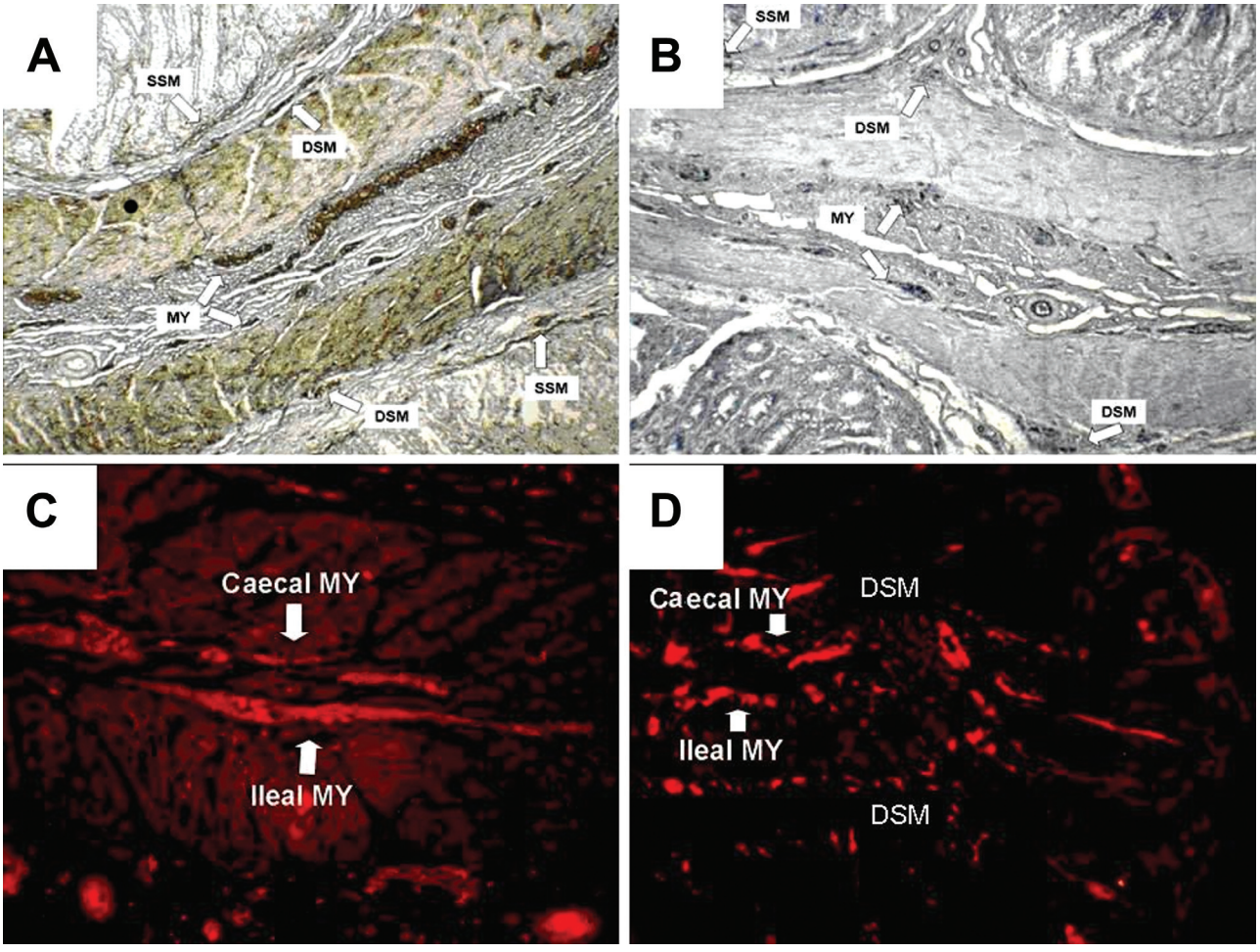

Fig. 3. This figure shows the ICV of a piglet. **A**: Transverse section stained with AchE histochemistry. **B**: Transverse section stained with NADPH-d histochemistry. **C**: Frontal section stained with PGP 9.5 fluorescent immunohistochemistry. **D**: Frontal section of the free end of the ICV stained with PGP 9.5 fluorescent immunohistochemistry. Note: there are two distinct ileal and cecal origins of the enteric nervous system are isolated along the ICV, but are connected at the free end of the valve. SSM, superficial submucous plexus; DSM, deep submucous plexus; MY, myenteric plexus

thickness along the valve, separated by a homogeneous longitudinal layer tapering gradually along the ICV.

Definite fibrous isolation has been reported between circular muscle layers of the gastroduodenal junction in guinea pigs by Cai et al. (1984) and in cats by Lammers AQ3 et al. (1998). In contrast, our results show that the circular muscle layers of the ileum and colon are not separated at the free end of the valve. This could explain the different motility patterns of the two junctions.

A study by Conklin and Du (1990) suggested that the ICC network is responsible for coordination of electrical activities along the axis of the colon. More recently, Wang et al. (2005) discovered a gap in the continuity in the myenteric ICC network at the pyloric sphincter, which is accountable for the break in the propagation of electric slow wave activity from the stomach to the duodenum and for the distinct uncoordinated peristaltic motor patterns of the stomach and the duodenum.

We have found that the ENS and myenteric ICC network have two distinct ileal and cecal origins in coaxial positions. However, these networks are isolated along the ICV by longitudinal muscle fibers and our findings clearly show that they are connected at the free end of the valve.

On the basis of our observations, it is easy to understand that the myoelectric slow wave activity can propagate from the ileum directly to the cecum, and ileal motor activities can pass through the ICV directly to the cecum irrelevant of propelled chyme, and can initiate cecal contractions as it was described by Malbert (2005). It is also possible that one of the cecal pacemakers found at the ICV by Shafik et al. (2005) corresponds to the propagating ileal myoelectric slow wave activity throughout the ICV.

The anatomy and neuroanatomy of the ileocecal junction showed in our study suggest that the ileocecal junction is a simple intussusception of the ileum into the cecum, which is in accordance with the latest concept of the ICV: intussusception of the terminal ileum into the cecum (Awapittaya et al., 2007).

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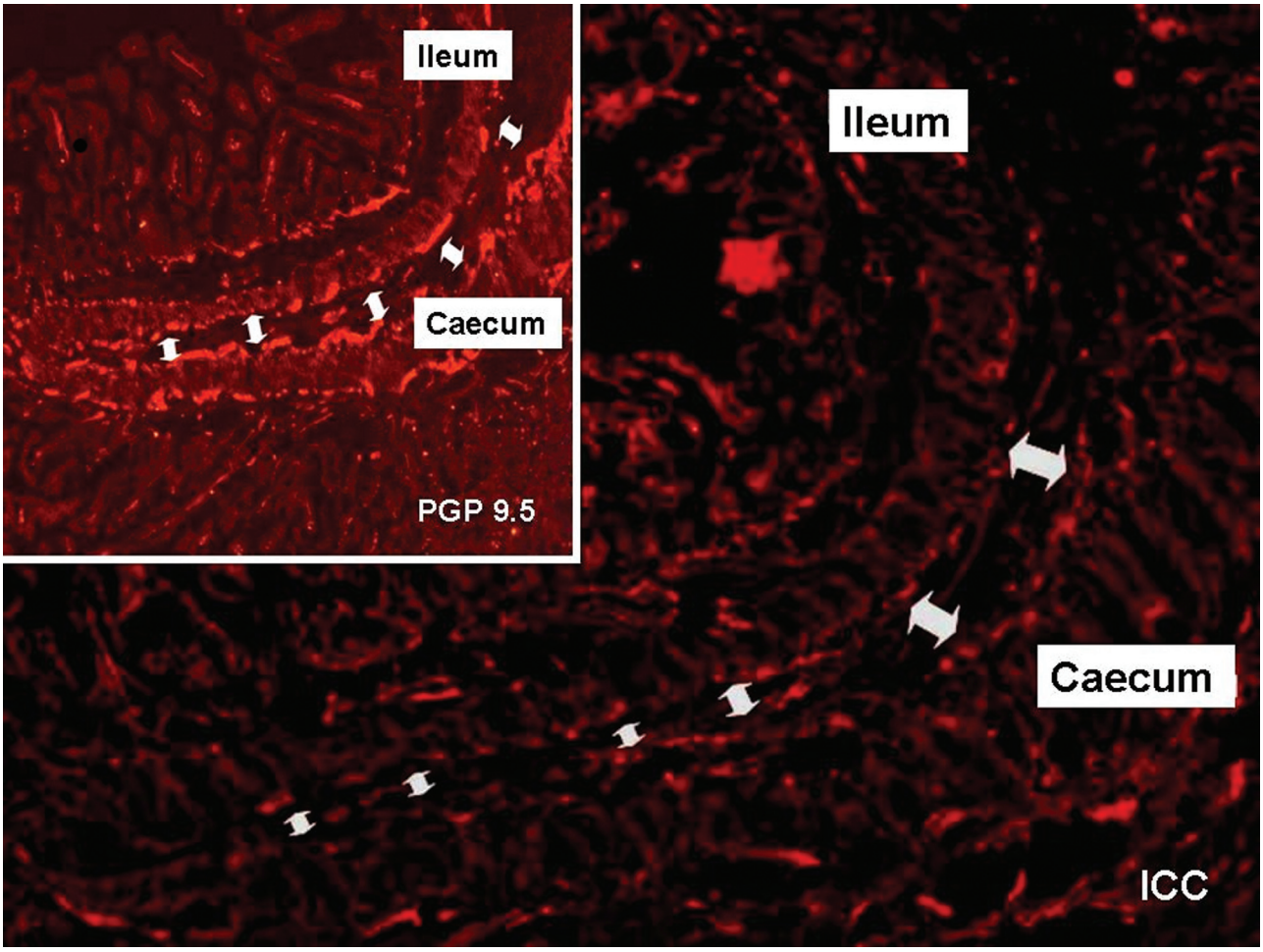

Fig. 4. Frontal section of piglet ICV. Left upper corner: PGP 9.5 fluorescent immunohistochemistry. Main picture: C-kit fluorescent immunohistochemistry. Note: Myenteric ICC network follows the myenteric plexus and it is continuous along the ICV. The double arrows point to the ileal and cecal myenteric plexuses on the image. The arrows are situated between the ileal and cecal myenetric plexuses either on the image of PGP 9.5 or on that of C-kit immunofluorescence staining.

The designs of previous techniques suggested for ICV replacement are not close to the original layout of the normal ileocecal junction (Fig. 5). For example, in the intussusception-like small bowel valve and in the Ricotta et al. (1981) valve technique, the created valve, the intussusceptum and the intususscipient, are both part of the small bowel. In the telescope anastomosis, reported by Zurita et al. (2004), the valve consists of only the wall of the ileum. In the ileocecal anastomosis suggested by Maegawa et al. (2005), the ileum is laid in a tunnel under the submucosa as it is in the Lich-Gregoire ureteral reimplantation. The wall of the valve consists of the ileum and the mucosa of the cecum.

On the basis of our observation, we propose a new ICV design: intussuscept part of terminal ileal segment together with proximal cecal wall and then perform an end-to-side ileocecal anastomosis (Fig. 5). This simple design will not recreate the apical continuity seen between the two myenteric plexuses of ICV; however, Horgan et al. (1993) observed regeneration of intramural autonomic nerves across the anastomotic scar, whether such fusion occurs in the ileocecal anastomosis with time need to be studied.

Probably, it cannot be expected that this new design will bring the efficacy of the surgically recreated valves up to the original's level alone, other external factors such as the role of the ileocecal ligament, reported by Kumar et al. (1988), have to be considered as well.

Physiological experiments by Conklin and Christensen (1975) revealed that circular muscle fibers of the ICV develop high tension in response to stretch in cats and opossum. Ouyang and Cohen (1981) and Bogers and Van Marck (1993) showed that these muscle fibers react differently to neurohumoral stimuli (e.g., CCK, gastrin, glucagon, secretin, acetylcholine, adrenoreceptor blockers) than the adjacent ileal and cecal muscle fibers. These observations suggest specific sphincteric properties of the circular muscle fibers of the ICV. Whether the fibers of the newly created valve will ever gain such an attribution remains a question.

In conclusion, the structure of the muscle layers and the ENS within the ICV supports a new concept: the ICV is a result of a simple intussusception of the terminal ileum into the cecum. This knowledge may help surgeons in their future attempts at reconstructing more

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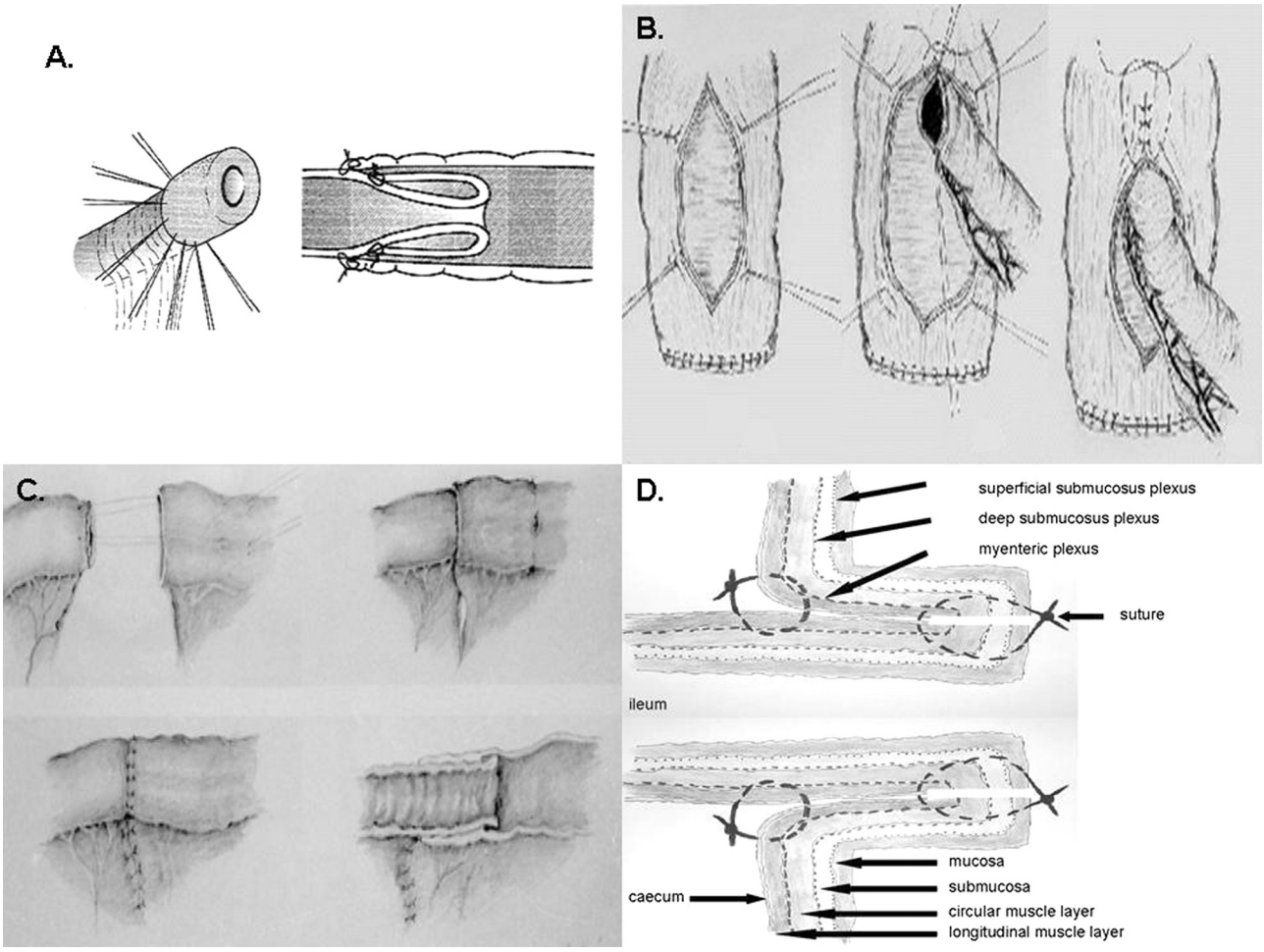

Fig. 5. Comparison of different ileocecal anastomosis techniques: **A**: Ricotta valve. **B**: Meagawa submucosal anastomosis. **C**: Zurita telescop anastomosis. **D**: New design of the ileocecal anastomosis by suturing the ileum end to the side of the cecum and telescoping the anastomosis into the cecum with 2nd layer serosal sutures

anatomically and functionally suitable ICVs following surgical resection of native ICVs.

LITERATURE CITED

Altdorfer K, Feher E, Feher J. 1996. Distribution and localization of nitric oxide containing neural elements in the digestive tract. Orv Hetil 137:857–860.

Awapittaya B, Pattana-arun J, Tansatit T, Kanjanasilpa P, Sahakijrungruang C, Rojanasakul A. 2007. A New concept of ileocecal junction: intussusception of the terminal ileum into the cecum. World J Gastroenterol 13:2855–2857.

Bakkevold KE. 2000. Nipple valve anastomosis for preventing recurrence of Crohn disease in the neoterminal ileum after ileocolic resection. A prospective pilot study. Scand J Gastroenterol 35:293–299.

Bogers JJ, Van Marck E. 1993. The ileocaecal junction. Histol Histopathol 8:561–566.

Cai WQ, Gabella G. 1984. Structure and innervation of the musculature at the gastroduodenal junction of the guinea-pig. J Anat 139:93–104.

Conklin JL, Christensen J. 1975. Local specialization at ileocecal junction of the cat and opossum. Am J Physiol 228:1075–1081.

Conklin JL, Du C. 1990. Pathways of slow-wave propagation in proximal colon of cats. Am J Physiol 258:G894–G903.

Cserni T, Szekeres JP, Furka I, Németh N, Józsa T, Mikó I. 2005. Hydrostatic characteristics of the ileocolic valve and intussuscepted nipple valves: an animal model. J Invest Surg 18:185–191.

DiDio LJA. 1968. Jejunum and ileum. In: The sphincters of the digestive system. Anatomical functional and surgical considerations. Baltimore: William and Wilkins. p 52–196. AQ4

Dinning PG, Bampton PA, Kennedy ML, Cook IJ. 1999. Relationship between terminal ileal pressure waves and propagating proximal colonic pressure waves. Am J Physiol 277:G983–G992.

Ecker KW, Pistorius G, Harbauer G, Feifel G. 1994. An intestinal neosphincter attained by circumscribed muscle proliferation. Technical development and functional evaluation in the dog. Langenbecks Arch Chir 379:361–367.

Ehrlein HJ, Schemann M. Gastrointestinal motility. Available at: http://www.wzw.tum.de/humanbiology/motvid01/tutorial.pdf. Accessed 15 June, 2007. AQ5

Hipper K, Ehrlein HJ. 2001. Motility of the large intestine and flow of digesta in pigs. Res Vet Sci 71:93–100.

Horgan AF, Molloy RG, Coulter J, Sheehan M, Kirwan WO. 1993. Nerve regeneration across colorectal anastomoses after low anterior resection in a canine model. Int J Colorectal Dis 8:167–169.

ID: kumaris Date: 23/11/08 Time: 12:16 Path: J:/Production/AR##/Vol00000/080206/3B2/C2AR##080206

Karnovsky MJ, Roots L. 1965. A direct colouring thiocholine method for cholinesterase. J Histochem Cytochem 12:219–221

Kaur U, Goyal N, Gupta M. 2005. Disposition of muscularis propria and nerve elements in the lip of the ileo-caecal valve. Nepal Med Coll J 7:125–128.

Kiernan JA. 1990. Histological and histochemical methods: theory and practice. New York: Pergamon Press.

Kumar D, Phillips SF. 1987. The contribution of external ligamentous attachments to function of the ileocecal junction. Dis Colon Rectum 30:410–416.

Kumar D, Phillips SF, Brown ML. 1988. Reflux from ileum to colon in the dog. Role of external ligamentous attachments. Dig Dis Sci 33:345–52.

Lammers WJ, Stephen B, Adeghate E, Ponery S, Pozzan O. 1998. The slow wave does not propagate across the gastroduodenal junction in the isolated feline preparation. Neurogastroenterol Motil 10:339–349.

Maegawa FA, de Souza JA, de Araujo EJ, Koh IH, d'Acampora AJ, de Farias DC, Mengarda J, Volpato D, da Silva LG, de Cordova CS. 2005. Ileocecal valve reconstruction in dogs. Acta Cir Bras 20:55–63.

Malbert CH. 2005. The ileocolonic sphincter. Neurogastroenterol Motil 17 (Suppl 1):41–49.

Ouyang A, Cohen S. 1981. Effects of hormones on gastrointestinal motility. Med Clin North Am 65:1111–1127.

Prado IM, Di Dio LJ. 2000. The "human"-like morphology of the swine papilla ilealis. Ann Anat 182:371–375.

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

Quigley EM, Thompson JS. 1994. Effects of artificial ileocolonic sphincter on motility in intestinal remnant following subtotal small intestinal resection in the dog. Dig Dis Sci 39:1222–1229.

Ricotta J, Zuidema GD, Gadacz TR, Sadri D. 1981. Construction of an ileocecal valve and its role in massive resection of the small intestine. Surg Gynecol Obstet 152:310–314.

Shafik A, El-Sibai O, Shafik AA. 2002. Physiological assessment of the function of the ileocecal junction with evidence of ileocecal junction reflexes. Med Sci Monit 8:629–635.

Shafik A, El-Sibai O, Shafik IA, Shafik AA. 2005. Identification of the location of the colonic pacemakers: a histologic study. Front Biosci 10:1661–1665.

Wang XY, Lammers WJ, Bercik P, Huizinga JD. 2005. Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine. Am J Physiol Gastrointest Liver Physiol 289:G539–G549.

Wilmore DW. 1972. Factors correlating with a successful outcome following extensive intestinal resection in newborn infants. J Pediatr 80:88–95.

Zurita M, Raurich JM, Ramirez A, Gil J, Darder J. 2004. A new neovalve type in short bowel syndrome surgery. Rev Esp Enferm Dig 96:110–118.

AQ1: Kindly check whether the short title is OK as given.

AQ2: Please note that the original structured and multiple paragraph abstract has been made into a destructured and set as a single paragraph per journal style.

AQ3: ''Cai et al., 1984'' is not given in the reference list. Kindly provide details of the same or delete the text citation.

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