

**Morphological Studies on the Wall Thickness and Mucous Cell
Distribution in the Rabbit Large Intestine**

(ウサギ大腸壁の厚さと粘液細胞の分布に関する形態学的研究)

2019

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ABBREVIATIONS

AB	Alcian blue
Ce	cecum
DC	distal colon
FC	fusus coli
HE	hematoxylin and eosin
PAS	periodic acid Schiff
P1	the first segment of the proximal colon
P1-h	the haustral part of P1
P1-t	the tenial part of P1
P2	the second segment of the proximal colon
P2-h	the haustral part of P2
P2-t	the tenial part of P2
Re	rectum

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are classified as hindgut fermenters and have a highly developed and well-differentiated large intestine. The rabbit cecum is a large organ having a wide bulge and a narrow constriction, both of which run spirally from the base of the cecum toward the appendix [20]. The colon is divided into the proximal and distal parts. The proximal colon consists of three segments; the first segment (P1) has three teniae that separate the rows of haustra, the second segment (P2) has a single tenia and one row of haustra, and the third segment called the fusus coli has no teniae and haustra [8, 15, 20, 21]. The mucosal surface of P1 and P2 exhibits a pattern of protrusions into the intestinal lumen, which are somewhat larger and more regularly arranged in P1 than P2 [20]. P1 also has more numerous tubular glands as compared to P2 [20]. The mucosal surface of the fusus coli is characterized by permanent longitudinally running folds [20], and displays the greatest density of tubular glands in its thick epithelium [20, 21]. Similarly, the distal colon and the rectum are also devoid of tenial and haustral structures. The distal colon has a smooth mucosal surface without any surface specialization and abundant mucous cells along short crypts [20]. The rectum that is next to the distal colon begins at the third sacral vertebral level and ends at the third coccygeal vertebral level, reaching the mean length of 7.2 cm [12].

For topographical descriptive purposes, traditional terms of the ascending, transverse and descending colon also exist. The ascending colon of the rabbit includes the proximal colon (P1, P2 and the fusus coli) and oral part of the distal colon, whereas the transverse and descending colons correspond to the aboral part of the distal colon. Since such traditional terminology does not fit with the functions played by each segment of the rabbit colon, "proximal and distal colon" is used more often than "ascending, transverse and descending colon" [20, 21].

Extensive physiological studies have shown that each segment of the rabbit large intestine displays unique and different functions during the formation of hard and soft feces [2, 7, 8, 10, 20]. The cecum receives the ileal contents and mixes them with the contents of the cecum for microbial fermentation [4]. P1 secretes fluid from the mucosa in order to dilute the digesta, and creates muscular contractions to facilitate mechanical separation of the digesta into small fermentable particles that are transported back to the cecum, and large unfermentable fibers that are passed toward P2 [4, 16]. P2 continues further mechanical separation of 2 kinds of particles, sending the small particles back to P1, and dividing the unfermentable particles into pellets to pass them anally [10]. During hard feces production, the *fusus coli* creates powerful contractions using the thick tunica muscularis in order to remove water out of the fecal pellets. The distal colon gradually absorbs water further from the fecal pellets and transfers them as hard, dry, round dark pellets to the rectum to be expelled from the anus [20, 21]. In contrast, during soft feces production, the *fusus coli* produces soft pellets by breaking down large boluses made by P2 that receives fermented cecal contents via P1 [10]. The *fusus coli* also envelopes soft pellets with a thick mucus sheath secreted by its mucosa in order to facilitate their speedy transport and to protect them against gastric digestion after cecotrophy [10]. The distal colon adds extra mucus to the envelope of the soft feces [5] and rapidly transfers them through the rectum to the anus [8, 21]. The studies that investigated the motility, secretion and absorption suggest that each segment of the rabbit large intestine, particularly the mucous and muscular layers, is important for the hard and soft feces formation. Although there have been a few fragmentary descriptions reported on the thickness of the entire wall in the cecum and of the mucosa in some segments [19, 20], there have been few comprehensive studies that have described in detail the thickness of the entire wall and constituent layers in each large intestinal segment. Similarly, elaborate descriptions of the distribution of the mucous cells in each segment are still insufficient. Therefore, the present

study measured the thickness of the wall and its constituent layers and investigated the distribution of mucous cells in each segment of the rabbit large intestine in order to find any possible correlation between microstructure and the functioning of each segment of the rabbit large intestine.

MATERIALS AND METHODS

All animal experimental procedures in the present study were approved (Approval No. H28-23) by the Research Ethics Committee for Animal Experimentation of the Tokyo University of Agriculture and Technology.

In this study, 3 healthy male New Zealand White rabbits (Tokyo Laboratory Animal Science Co., Tokyo, Japan) aged 10–11 weeks (2.0–2.1 kg) were used. Until the day of sampling, all animals were kept in individual cages in an air-conditioned room with controlled temperature and free access to food and water. Prior to sacrifice, the animals were sedated with xylazine (10 mg/kg, intramuscular) and then euthanized with sodium pentobarbital (100 mg/kg, intravenous or intraperitoneal). The abdominal cavity was opened along the linea alba in order to carefully separate the large intestine from the mesentery. Samples with 2 cm length along the oro-anal axis were collected at the following sites for each segment; for the cecum, the bulging wall in the middle part along the proximodistal axis of the cecal second gyrus; for P1, 4 cm anal to the ceco-colic border; for P2, 9 cm anal to the P1-P2 border; for fusus coli, 2 cm anal to the P2-fusus coli border; for the distal colon, 40 cm anal to the fusus coli-distal colon border; and finally, for the rectum, 4 cm oral to the anus (Fig. 1). Immediately after the samples were gently washed with cold saline to remove the residues of intestinal contents, they were fixed by immersion into 4% paraformaldehyde in 0.1 M phosphate buffer for 24 hr at 4°C. Subsequently, the samples were then embedded in paraffin and cut transversely at a 6–8 μ m thickness. The sections were processed with conventional hematoxylin and eosin (HE) to observe the general laminar structure and measure the thickness. Other sections were stained with Alcian blue (AB) pH 2.5, or periodic acid Schiff (PAS) or combined AB and PAS, followed by hematoxylin, to observe the distribution of mucous cells. Digital images of representative sections were taken using a DS-Ri1 camera (Nikon, Tokyo, Japan) attached to an Eclipse Ni-U microscope (Nikon). The

images were adjusted to obtain adequate color, sharpness, brightness and resolution by Adobe Photoshop (Adobe Systems, San Jose, CA, USA), and assembled into Adobe Illustrator CC (Adobe Systems).

For quantitative analysis, measurements were obtained from HE-stained sections of the bulging part in the cecum, of the tenial and haustral parts in P1 and P2, and of the non-folded part in the fusus coli, distal colon and rectum. From each of these sections, 6 sites were randomly selected to measure the thickness of the entire intestinal wall and its constituent layers (mucosa, lamina muscularis mucosae, submucosa, inner circular and outer longitudinal layer of the tunica muscularis, and serosa or adventitia), using ImageJ version 1.15s software (NIH, Bethesda, ML, USA). The thickness of the mucosa included the lamina propria. To compare the differences in the thickness between each part of the segment across 3 rabbits, the data were assembled, and the means and standard deviations were calculated using Excel version 16.20 software (Microsoft, Redmond, WA, USA). Subsequently, the difference in the total and laminar thicknesses was statistically tested by either a one-way analysis of variance and the Tukey's test for comparison of more than 2 means or Student's *t* test for comparison of 2 means, using Prism 8 version 8.1.1 software (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered to be statistically significant.

For number of mucous cells, it is evaluated as "a few", "a moderate number", "numerous" and "the most numerous", when the number of mucous cells fell into <11 , $11-50$, $51-100$, and $100<$, respectively, within the $50\ \mu\text{m}$ -width mucosa in randomly selected 6 sites of each part whose mucous cells were stained.

RESULTS

Wall structure and thickness

Figures 2-5 and Tables 1-3 present the structural characteristics and thickness measurements of the wall and its constituent layers in each segment of the large intestine. Observation of the HE-stained sections showed that the cecum had the thinnest entire wall, mucosa and tunica muscularis (Fig. 2a). P1 had a thicker entire wall and a more well-developed mucosa than the cecum (Fig. 2b and c). Furthermore, the tenial part of P1 had thicker inner circular and outer longitudinal layers of the tunica muscularis than the P1 haustral part, whose outer longitudinal layer was almost invisible (Fig. 2b and c). In the tenial and haustral part of P2 (Fig. 2d and e), the entire wall was thinner than in the corresponding part of P1. The mucosa in the tenial part of P2 was somewhat more well-developed than that in the haustral part. The tunica muscularis in P2 was more well-developed in the tenial part than the haustral part, whose outer longitudinal layer was again almost invisible (Fig. 2d and e). In the fusus coli (Fig. 2f), the mucosa was the thickest throughout the large intestine and the tunica muscularis was relatively well-developed. The distal colon, particularly its mucosa, was thinner than the fusus coli (Fig. 2g), and the rectum had the thickest entire wall, lamina muscularis mucosae and tunica muscularis across the large intestine (Fig. 2h).

Quantitative analysis more clearly showed the difference in the thickness of the entire wall and the constituent layers of each segment. The entire intestinal wall in the cecum was significantly thinner ($299.7 \pm 32.8 \mu\text{m}$) and that in the fusus coli ($996.9 \pm 168.0 \mu\text{m}$) and rectum ($1048.1 \pm 108.4 \mu\text{m}$) was significantly thicker, compared to the other segments (Fig. 3a and Table 1). In-between were P1 ($680.6 \pm 117.0 \mu\text{m}$), P2 ($676.7 \pm 123.4 \mu\text{m}$) and the distal colon ($626.4 \pm 115.9 \mu\text{m}$), whose thickness of the entire wall did not significantly differ from each other (Fig. 3a and Table 1).

For the thickness of the mucosa (Fig. 3b and Table 1), the cecum was significantly thinner ($136.8 \pm 23.0 \mu\text{m}$) and the fusus coli was significantly thicker ($627.7 \pm 129.9 \mu\text{m}$) as compared to the other segments. Moreover, P1 had a significantly thicker mucosa, compared to all of the segments other than the fusus coli (Fig. 3b).

For the lamina muscularis mucosae (Fig. 3c and Table 1), the thickness did not differ among the cecum ($7.5 \pm 2.4 \mu\text{m}$), P1 ($11.5 \pm 6.1 \mu\text{m}$) and P2 ($12.3 \pm 4.3 \mu\text{m}$), all of which had a significantly thinner lamina muscularis mucosae than the distal colon and rectum. The cecum and P1 also had a significantly thinner lamina muscularis mucosae than the fusus coli (Fig. 3c). The fusus coli ($20.8 \pm 16.2 \mu\text{m}$) had a significantly thinner lamina muscularis mucosae than the distal colon ($54.2 \pm 9.8 \mu\text{m}$), which, in turn, had a significantly thinner lamina muscularis mucosae than the rectum ($114.6 \pm 20.9 \mu\text{m}$) (Fig. 3c and Table 1).

The thickness of the submucosa did not have any significant difference among others in the cecum ($33.1 \pm 13.5 \mu\text{m}$), P1 ($34.5 \pm 17.9 \mu\text{m}$), P2 ($37.1 \pm 28.7 \mu\text{m}$), the fusus coli ($41.5 \pm 23.0 \mu\text{m}$) and the distal colon ($49.2 \pm 12.0 \mu\text{m}$) (Fig. 3d and Table 1). However, the rectum ($72.3 \pm 31.3 \mu\text{m}$) had a significantly thicker submucosa (Fig. 3d and Table 1), compared to all of the other segments.

For both layers of the tunica muscularis, the cecum was significantly thinner ($63.8 \pm 18.9 \mu\text{m}$ for the inner circular and $41.9 \pm 10.4 \mu\text{m}$ for the outer longitudinal layer) in comparison to the other segments except for the outer longitudinal layer of the distal colon (Fig. 3e and f and Table 1). On the contrary, both layers of the rectum were significantly thicker ($350.8 \pm 80.7 \mu\text{m}$ for the inner circular and $208.6 \pm 38.5 \mu\text{m}$ for the outer longitudinal layer), in comparison to those of the other segments (Fig. 3e and f and Table 1). There was a second peak of the thickness of the tunica muscularis in the proximal colon along the oro-anal axis of the large intestine (Fig. 3e and f and Table 1), where P2 ($255.5 \pm 72.4 \mu\text{m}$) was the second thickest for the inner circular layer (Fig. 3e and Table 1), whereas

P1 ($117.7 \pm 64.2 \mu\text{m}$) was the second thickest for the outer longitudinal layer (Fig. 3f and Table 1).

Finally, the cecum ($16.6 \pm 6.7 \mu\text{m}$), P1 ($18.0 \pm 7.7 \mu\text{m}$), P2 ($14.9 \pm 9.6 \mu\text{m}$) and the rectum ($22.4 \pm 8.8 \mu\text{m}$) had significantly or tended to have a thicker serosa than the fusus coli ($9.6 \pm 9.5 \mu\text{m}$) and distal colon ($7.1 \pm 4.3 \mu\text{m}$) (Fig. 3g and Table 1).

The thickness of the entire wall and its constituent layers was also compared between the tenial and haustral part of P1 (Fig. 4 and Table 2) and between the tenial and haustral part of P2 (Fig. 5 and Table 3). For P1, the tenial part ($765.1 \pm 92.2 \mu\text{m}$) had a significantly thicker entire wall than the haustral part ($596.1 \pm 67.7 \mu\text{m}$) (Fig. 4a and Table 2). The inner circular and outer longitudinal layers were significantly thicker in the tenial ($143.9 \pm 22.9 \mu\text{m}$ and $152.3 \pm 22.9 \mu\text{m}$, respectively) than in the haustral part ($120.3 \pm 31.9 \mu\text{m}$ and $14.2 \pm 4.3 \mu\text{m}$, respectively) (Fig. 4e, f, and Table 2), whereas the serosa was significantly thicker in the haustral ($19.9 \pm 9.8 \mu\text{m}$) than the tenial part ($16.0 \pm 4.2 \mu\text{m}$) (Fig. 4g and Table 2). The thickness of the mucosa, lamina muscularis mucosae and submucosa did not have any significant difference between the tenial and haustral part (Fig. 4b, c and d, and Table 2).

For P2, there was no significant difference in the thickness of the entire wall between the tenial ($715.0 \pm 132.0 \mu\text{m}$) and haustral part ($638.4 \pm 104.0 \mu\text{m}$) (Fig. 5a and Table 3). However, the mucosa was significantly thicker in the tenial part ($333.2 \pm 70.8 \mu\text{m}$) than in the haustral part ($282.4 \pm 46.4 \mu\text{m}$) (Fig. 5b and Table 3), whereas the inner circular layer was significantly thinner in the tenial part ($219.6 \pm 53.5 \mu\text{m}$) than in the haustral part ($291.5 \pm 72.1 \mu\text{m}$) (Fig. 5e and Table 3). The outer longitudinal layer of the tunica muscularis was observed only at 1 out of 18 sites in the haustral part, therefore, statistical test was not able to be performed. There were no significant differences in the thickness of the lamina muscularis mucosae, submucosa and serosa between the tenial and haustral part (Fig. 5c, d and f, and Table 3).

Mucous cell distribution

Sections stained with AB, PAS and AB-PAS showed that the distribution of mucous cells was remarkably different along the oro-anal axis of the large intestine (Figs. 6 and 7). In general, mucous cells were the fewest in the cecum and most numerous in the fusus coli. Most of the individual mucous cells were stained with both AB and PAS, unless otherwise mentioned below.

The cecum had a few mucous cells, which rarely made clusters and resided in the epithelium facing the intestinal lumen and lining the shallow intestinal crypt (arrowheads in Fig. 6a, a' and a"). In P1, the staining pattern was similar in the tenial and haustral parts. Numerous mucous cells were present in the epithelium lining the crypt, particularly in the deep half (Fig. 6b, b' and b"), and a small number of mucous cells were also located in the surface epithelium. There was a tendency that cells stained with AB alone were distributed more near the neck of the crypt (Fig. 6b and b"), while those stained with PAS alone were distributed more in the bottom of the crypt (Fig. 6b' and b"). There was another tendency that cells stained with PAS were darker in the deep crypt than in the superficial crypt and surface epithelium. In P2, the staining pattern was similar in the tenial and haustral parts. Mucous cells in P2 were also numerous and were distributed in a manner similar to those in P1 (Fig. 6c, c' and c"). In the thick mucosa of the fusus coli, mucous cells were the most numerous and tended to be aggregated in the epithelium that lined the deep half of the intestinal crypt, while they were somewhat more diffusely distributed in the epithelium that lined the superficial half of the crypt (Fig. 7a, a' and a"). A small number of mucous cells were also located in the surface epithelium. The distal colon and rectum (Fig. 7b, b', b", c, c' and c") had a moderate number of mucous cells that were aggregated in the epithelium lining the crypt, while the surface epithelium had fewer, scattered mucous cells. Mucous cells stained with PAS were distributed more densely in the distal colon than the rectum (Fig. 7b' and c'), however, these cells

were fewer than the mucous cells stained with AB in both the distal colon and rectum (Fig. 7b, b', c and c').

DISCUSSION

This is the first systematic study documenting the thickness of the wall and the mucous cell distribution throughout all segments of the rabbit large intestine. The present findings demonstrated that the cecum had the thinnest entire wall across the large intestine. The thickness that observed in the present study ($299.7 \pm 32.8 \mu\text{m}$) was remarkably thinner as compared to the $554.5 \mu\text{m}$ thickness reported by Snipes [19]. The reason for the difference between the findings of Snipes [19] and the current study may be attributable to several factors such as differences in the strain, age, and numbers of the rabbit, in addition to the sampling sites, tissue processing, and measurement methods. This may also explain the difference in the thickness values of other segments between the findings of Snipes and the present study [19, 20]. The cecum has the thinnest mucosa in agreement with a previous study [19], and the mucosal epithelium contains only a few mucous cells. This indicates that most of the epithelial cells may consist of absorptive cells, which are advantageous for absorption of electrolytes and volatile fatty acids through the cecal mucosa [6, 8, 15]. The cecum also has the thin tunica muscularis, which does not create so strong contraction of the wall [18]. It is, therefore, suggested that the ingesta in the cecum may be gently mixed for fermentation that takes place within the cecum.

The present findings showed that the entire wall of P1 was as thick as that of P2 and the distal colon. Within P1, the tenial wall is thicker than the haustral wall. This is attributable to the thicker inner circular and outer longitudinal layers of the tunica muscularis in the tenial part and the occasional absence of the outer longitudinal layer in the haustral part. The mucosa of P1 was the second thickest

across all of the segments, in agreement with the results reported by Snipes et al. [20]. In the mucosa, numerous mucous cells located in the crypt may add mucus to lubricate the transport of intestinal contents going back to the cecum and forward to P2 [3, 10]. The mucous cells stained with AB alone tended to be arranged more superficially in the crypt than those stained with PAS alone. This arrangement appears beneficial to resist against microbial invasion through the epithelium, since acidic mucins secreted by AB-stained mucous cells may be resistant to microbial degradation of the intestinal mucosa [9, 17]. The paucity of mucous cells in the surface and superficial crypt epithelium, that is, rich absorptive cells in these sites, may be advantageous for effective electrolyte absorption [20]. Also of particular interest is that P1 has a well-developed longitudinal outer muscular layer of the tunica muscularis, but has a poorly developed circular layer, particularly in the haustral part. This muscular arrangement may be important to facilitate separation of digesta into small fermentable particles and unfermentable fibers for soft and hard feces formation, although the exact mechanisms of such separation are still unknown.

The mucosa in P2 was somewhat thinner than that in P1. Within P2, the mucosa of the tenial part was thicker than that of the haustral part. The distribution of mucous cells in both parts of P2 was essentially similar to that in P1. For the tunica muscularis, the inner circular layer in P2 was the second thickest across the large intestine. Since P2 is the only segment where the formation of hard fecal pellets takes place [10], it is considered that the presence of the thick circular layer in P2 is important for creating strong segmental contractions in order to separate the solid contents into pellets, and for carrying the liquid contents back towards the cecum [10].

The fusus coli had the thickest entire wall among each segment of the large intestine, except for the rectum that was as thick as the fusus coli. The thickness of the fusus coli was due to the thickest mucosa across the large intestine. The data of mucous cell-staining demonstrated that the fusus coli had the most

numerous mucous cells compared to the other segments. The mucus secreted by these cells may lubricate the mucosal surface for facilitation of speedy transport of soft fecal pellets and to enwrap them to be protected against digestion after cecotrophy [20, 21]. The paucity of the mucous cells in the superficial part of the crypt epithelium indicates that this part may contain more absorptive cells, which may work to remove water out of the fecal pellets [20, 21]. In the fusus coli, the circular and longitudinal layers of the tunica muscularis were relatively well-developed. It is assumed that the contraction of the tunica muscularis of the fusus coli forms the small soft fecal pellets. In addition, it has also been postulated that during the hard feces formation, the contraction of the tunica muscularis may extrude water out of the hard pellets [10, 20].

The entire wall of the distal colon is relatively well-developed. In the mucosa, the mucous cells stained with AB was more numerous than those stained with PAS. As discussed above, this may be beneficial to protect the mucosa against microbial invasion [9, 17]. The distal colon has a thick inner circular layer of the tunica muscularis relative to the outer longitudinal layer. This may be advantageous for speedy transport of soft feces towards the anus and extrusion of water from hard feces, as the fusus coli may also do [10, 21].

The rectum was shown to have the thickest entire wall across all of the other segments except for the fusus coli. In the mucosa, the distribution of the mucous cells in the rectum was similar to that in the distal colon. The rectum had the thickest lamina muscularis mucosae and circular and longitudinal layers of the tunica muscularis across all of the other segments. In rabbits, soft feces are expelled once or twice a day while hard pellets are often expelled throughout the day [21]. Therefore, the presence of a thick lamina muscularis mucosae and tunica muscularis in this segment may be an advantageous benefit for adapting to frequent defecation.

This study demonstrated that the thickness of the wall and its layers as well as the distribution of mucous cells considerably vary between each segment of

the rabbit large intestine. The thickness measurements indicate that the difference is more pronounced in the tunica mucosa and tunica muscularis across the large intestine. It is believed that the presence of such structural differences in each segment of the large intestine is essential for the formation of soft and hard feces in rabbits. Therefore, the present findings are important as a basis for understanding physiology of the rabbit large intestine and its pathological conditions, such as bowel inflammatory diseases [1, 11] and intestinal microbial infections [13, 14], where the normal structure of intestine may be altered.

SUMMARY

To achieve a better understanding of rabbit large intestinal functions, such as production of hard and soft feces and cecal fermentation, knowledge of the intestinal wall structure is essential. However, such knowledge is far from complete. Therefore, the aims of this study were to measure the thickness of the wall and its constituent layers and describe distribution of mucous cells in each segment of the large intestine in New Zealand White rabbits. Results showed that the cecum had the thinnest entire wall throughout the large intestine, and the fusus coli and rectum had a thicker entire wall in comparison to the cecum, the first segment of the proximal colon, the second segment of the proximal colon, and the distal colon. Moreover, the thickness of the mucosa in the fusus coli and that of the inner and outer layers of the tunica muscularis in the rectum were greater than that of the other segments. Mucous cells in the mucosa were the fewest in the cecum and most numerous in the fusus coli. This study provides detailed knowledge of the wall thickness and distribution of mucous cells in the large intestine of the rabbit. These findings are important for improving our understanding of rabbit intestinal physiology and pathology.

ACKNOWLEDGEMENT

First of all, I would like to sincerely thank The Omniscient and The Merciful (Allah) who blessed and made me capable enough to overcome all the challenges, to reach this phase of my life, and to write this dissertation.

This dissertation is the result of a quest for knowledge that started more than 4 years ago, and I would like to thank each and every person who guided, encouraged or inspired me during these years. My special gratitude goes to:

My main advisor Professor **Hideshi SHIBATA** at Tokyo University of Agriculture and Technology (TUAT), I admire your knowledge, enthusiasm and I am grateful that you believed in me and accepted me as your student. Thank you for your never ending support, constructive guidance and patience. You taught me to think and work independently, encouraged my ideas and motivated me.

My co-advisors Professor **Gen WATANABE** at TUAT, Associate Professor **Nobuaki NAKAMUTA** at Iwate University, Professor **Nobuo KITAMURA** at Obihiro University of Agriculture and Veterinary Medicine, and Associate Professor **Shouichiro SAITO** at Gifu University, thank you for your help and support.

Associate Professor **Masahiro KANEDA** at TUAT, thank you for constant encouragement and support.

All the past and present members of my lab for their kind assistance and encouragement.

Tetsuhito KIGATA, thank you for being a wonderful friend and great colleague. For all the guidance and help with the Ph.D. rules during these years. I wish you a lot of success in the rest of your career.

Reona IKEGAMI, for being a kind tutor, helping me with the paper works, and making my first year of stay comfortable.

Mohi UDDIN and all the other lab members, for your continuous support and encouragement.

I feel thankful to the **Japanese government** for providing me the scholarship and the chance to pursue my studies at UGSVS, TUAT.

My beloved late **father**, for your immense support and encouragement. Your loss was extremely hard to me, and I feel happy that by getting my Ph.D. degree I can make at least one of your wishes come true.

My beloved **mother**, for your prayers, support and continuous guidance, and my **sisters and brothers** for your constant encouragements and best wishes.

A huge thanks to my wonderful wife **Fatuma** and lovely children **Ali Yousuf** and **Sahar**, without your support I could never finish this dissertation. **Fatuma** was always there, listening to me, supporting me, and encouraging me. Thanks for undertaking all the family responsibilities, taking care of our lovely children, and allowing me to concentrate and spent much of my time on my research and writing this dissertation.

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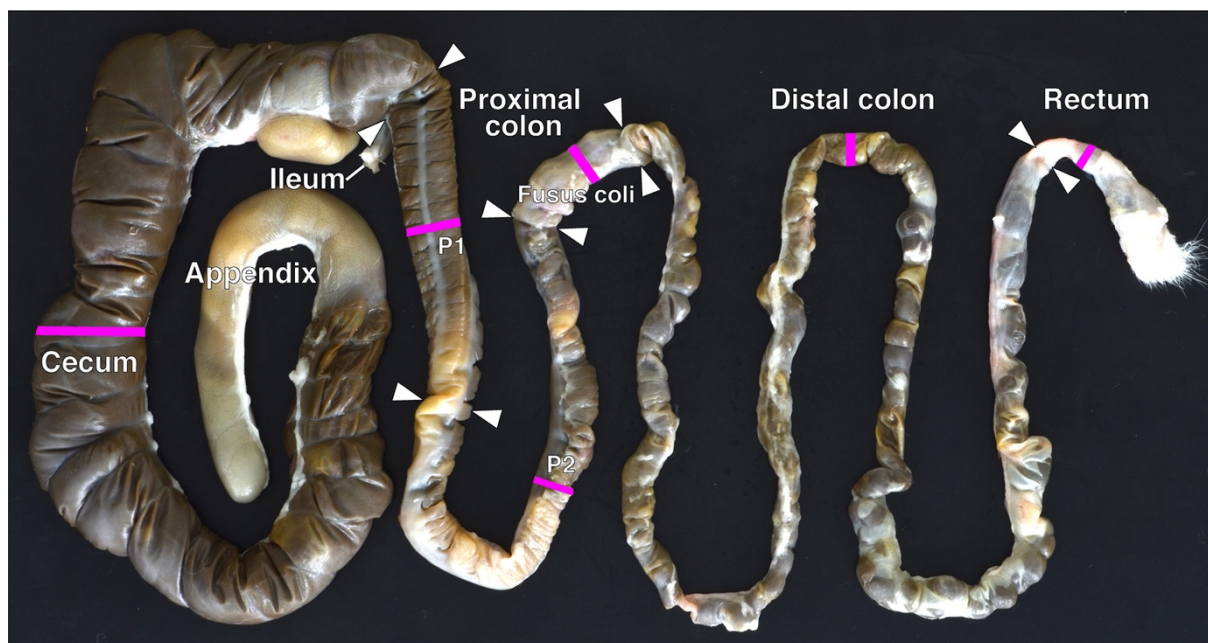
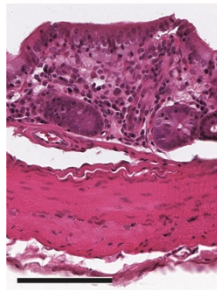


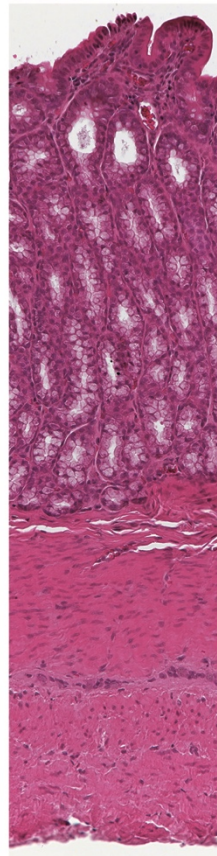
Fig. 1. Photograph showing approximate sampling sites (magenta bars) depicted in an unfolded rabbit large intestine. Arrowheads indicate the borders of each segment.

Fig. 2. Cross sections of the cecum (a), tenial part of P1 (b), haustral part of P1 (c), tenial part of P2 (d), haustral part of P2 (e), fusus coli (f), distal colon (g) and rectum (h), demonstrating the difference in the thickness of the entire wall and the constituent layers between each segment. HE staining. Scale bar, which is applicable to all panels, equals 100 μm .

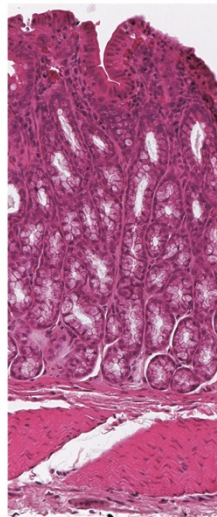
(a) Cecum



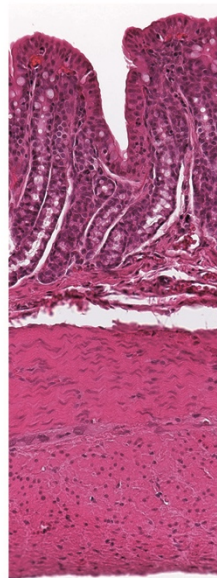
(b) P1, tenial



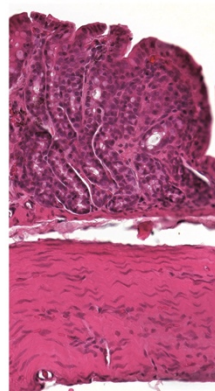
(c) P1, haustral



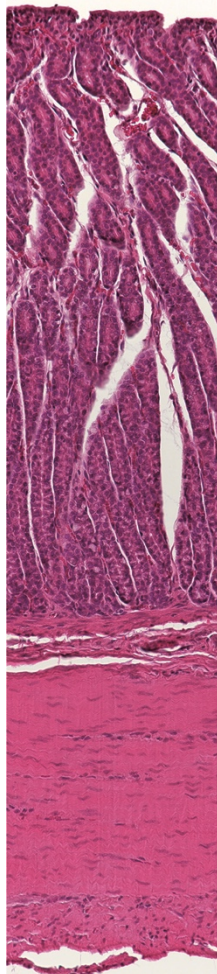
(d) P2, tenial



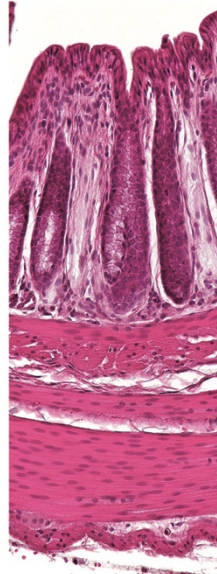
(e) P2, haustral



(f) Fusus coli



(g) Distal colon



(h) Rectum

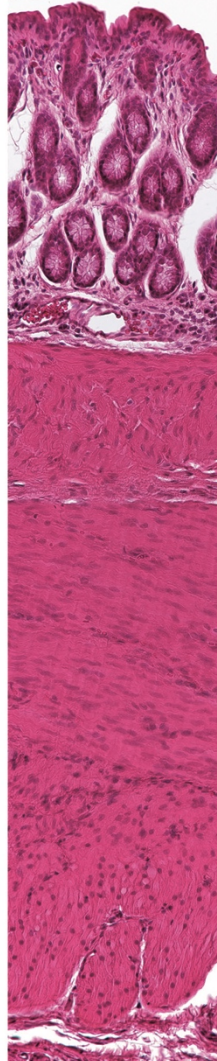


Fig. 3. Columnar graphs showing the statistical difference in the thickness of the entire wall (a), mucosa (b), and lamina muscularis mucosae (c), submucosa (d), inner circular layer of the tunica muscularis (e), outer longitudinal layer of the tunica muscularis (f) and serosa (g) between each segment across 3 rabbits. The height and whisker of each column represent the mean \pm SD at $n=18$ for the cecum, fusus coli, distal colon, and rectum, and $n=36$ for P1 and P2. The X-axis indicates the intestinal segment, whereas the Y-axis indicates the thickness in μm . *, $P<0.05$. Ce, cecum; DC, distal colon; FC, fusus coli and Re, rectum. Note that for the rectum in (g), the value is of the adventitia.

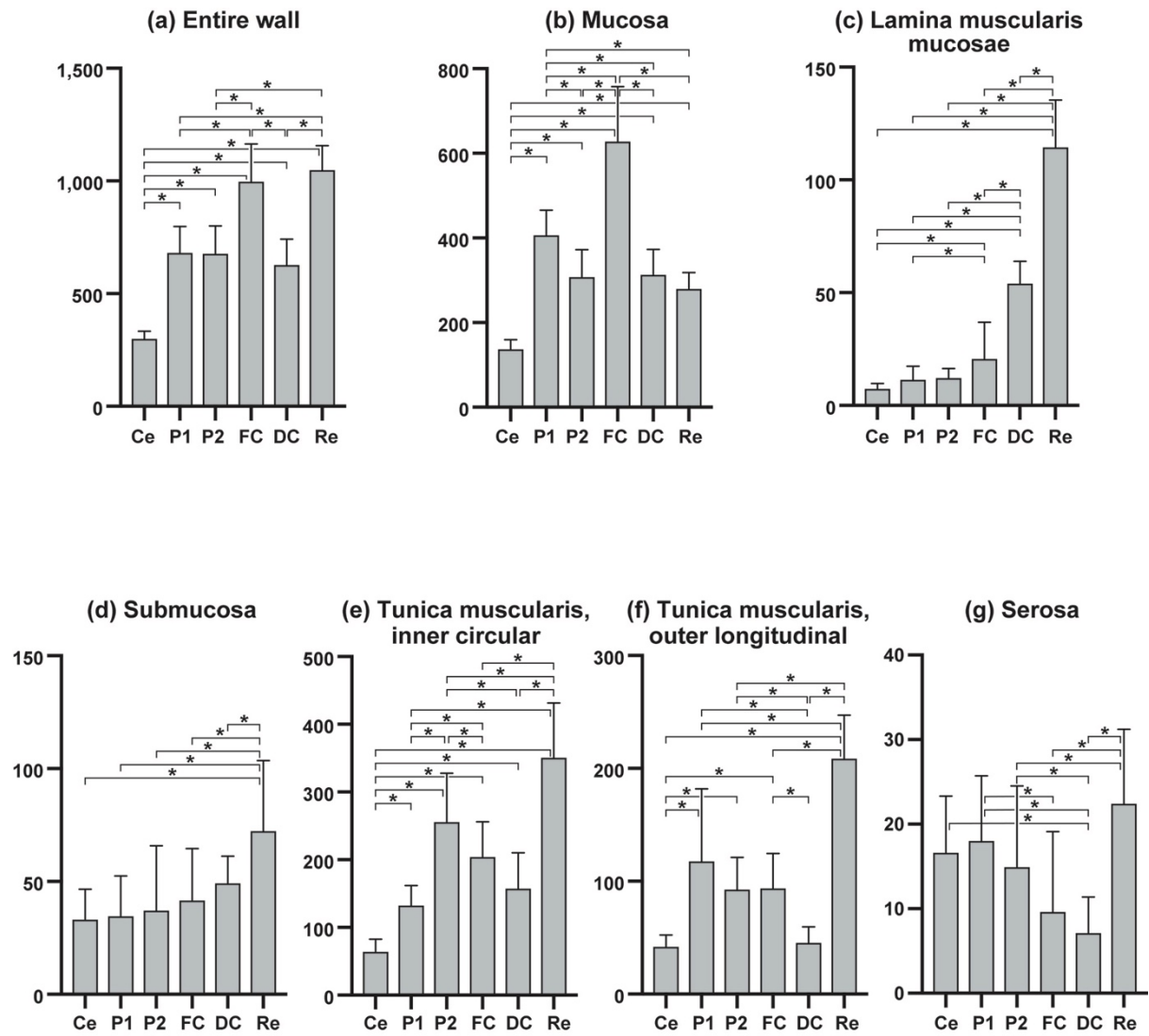


Fig. 4. Columnar graphs showing the statistical difference in the thickness of the entire wall (a), mucosa (b), lamina muscularis mucosae (c), submucosa (d), inner circular layer of the tunica muscularis (e), outer longitudinal layer of the tunica muscularis (f) and serosa (g) between the tenial and haustral part of P1 across 3 rabbits. The height and whisker of each column represent the mean \pm SD. $n=18$ except for the outer longitudinal layer of the haustral part, where $n=6$. The X-axis indicates the intestinal segment, whereas the Y-axis indicates the thickness in μm . *, $P<0.05$. P1-t, the tenial part of P1 and P1-h, haustral part of P1.

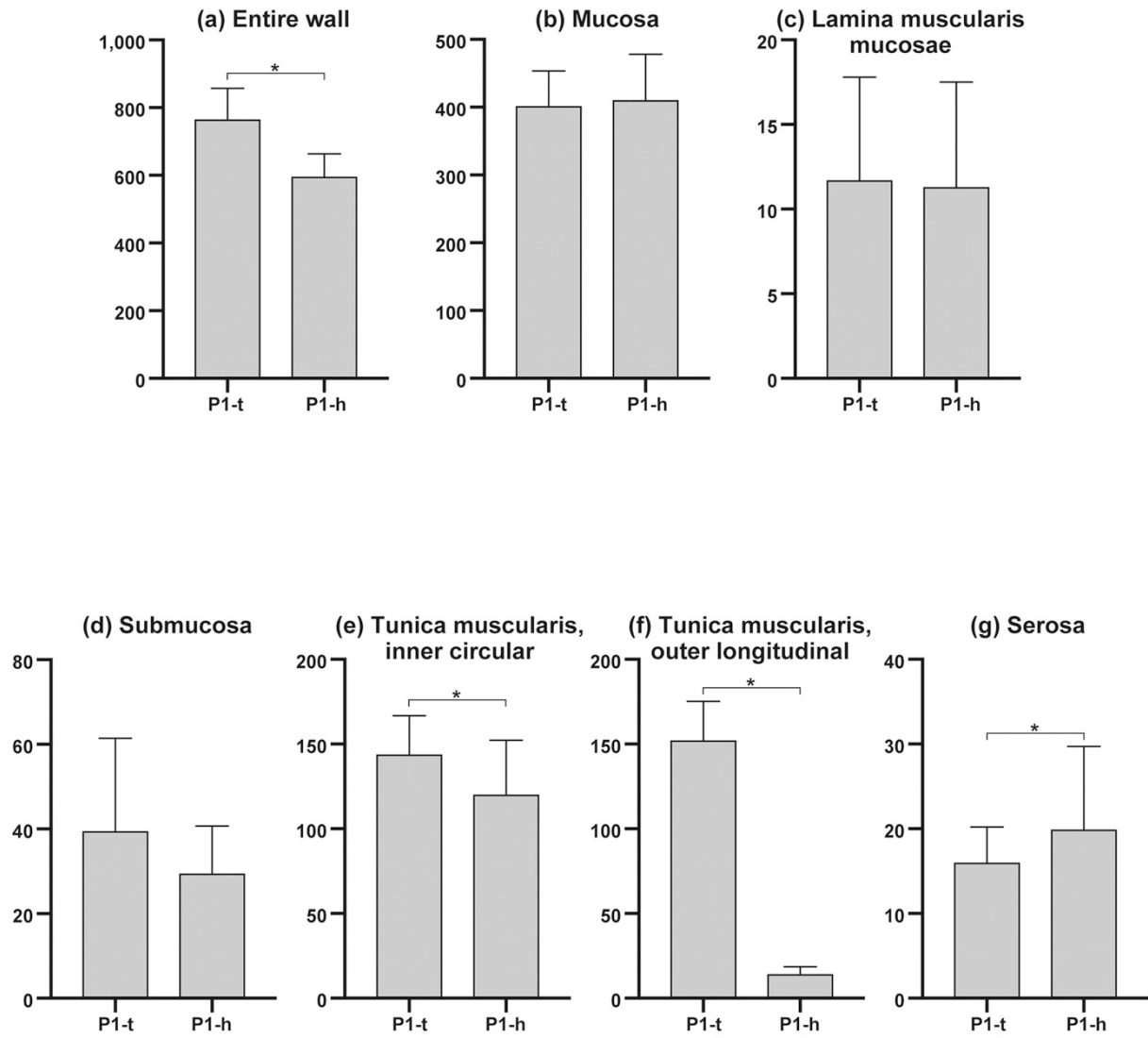


Fig. 5. Columnar graphs showing the statistical difference in the thickness of the entire wall (a), mucosa (b), lamina muscularis mucosae (c), submucosa (d), inner circular layer of the tunica muscularis (e) and serosa (f) between the tenial and haustral part of P2 across 3 rabbits. The height and whisker of each column represent the mean \pm SD. $n=18$. The X-axis indicates the intestinal segment, whereas the Y-axis indicates the thickness in μm . *, $P<0.05$. P2-t, the tenial part of P2 and P2-h, haustral part of P2.

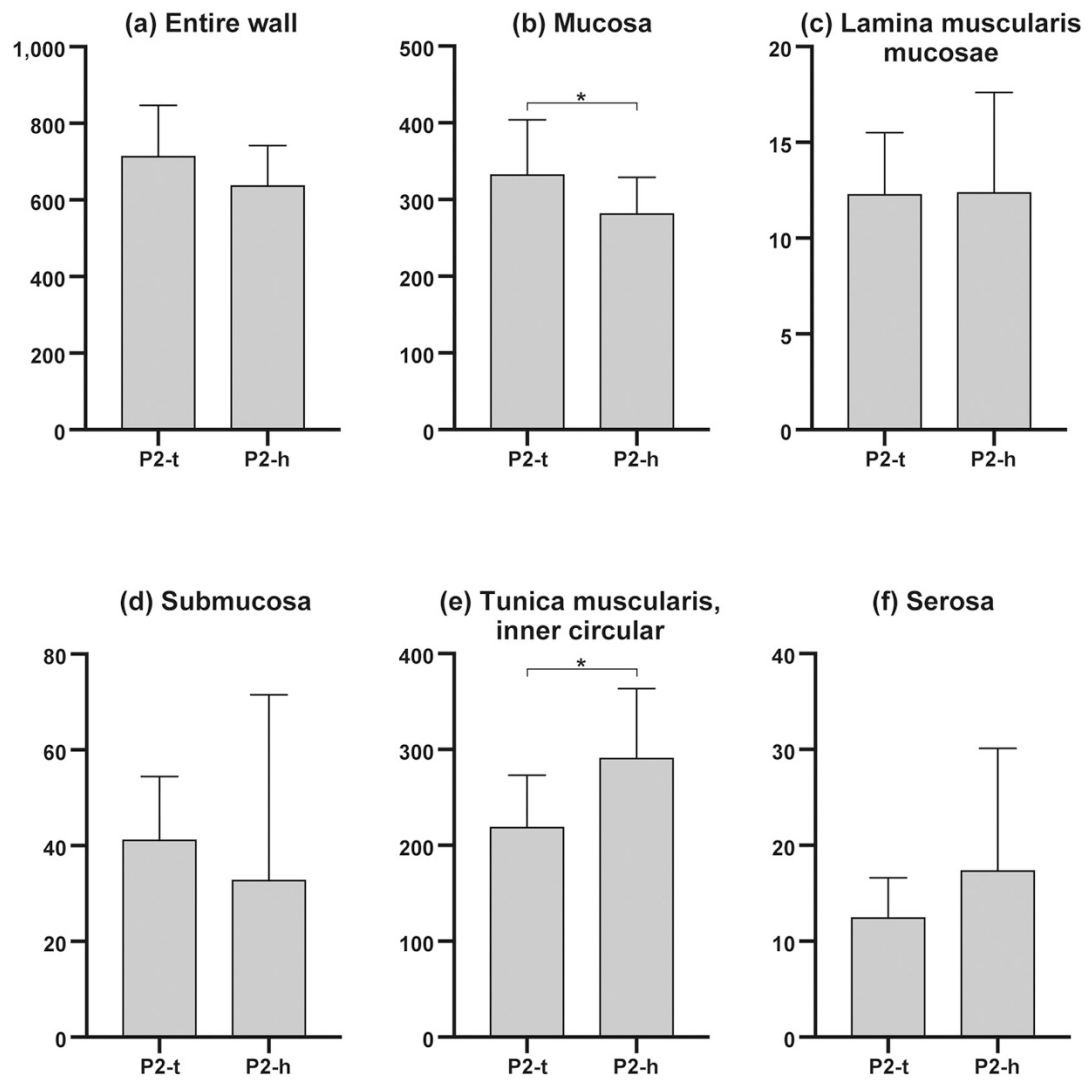
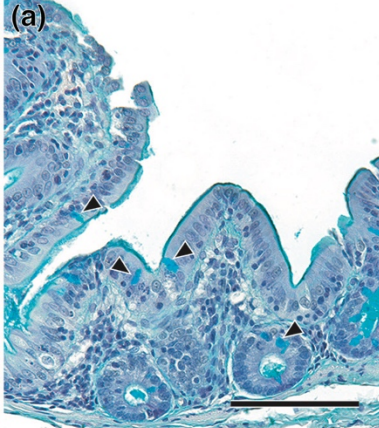
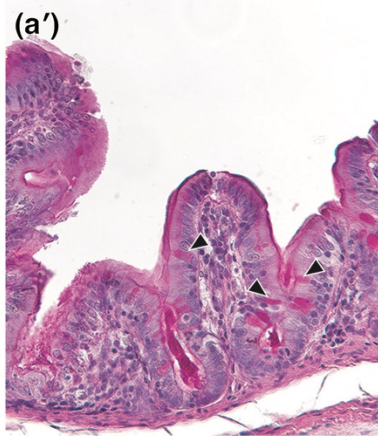


Fig. 6. Cross sections of the mucosa of the cecum (a, a' and a''), tenial part of P1 (b, b' and b'') and tenial part of P2 (c, c' and c''), demonstrating the difference in the distribution of mucous cells in each segment. Alcian blue (a, b, and c), periodic acid Schiff (a', b' and c') and combined Alcian blue-periodic acid Schiff staining (a'', b'' and c''). Arrowheads in (a), (a') and (a'') indicate a few mucous cells. Scale bar, which is applicable to all panels, equals 100 μm .

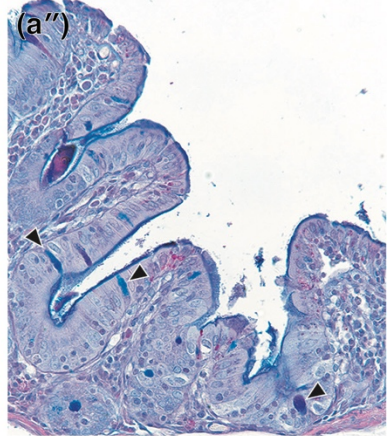
Cecum
(a)



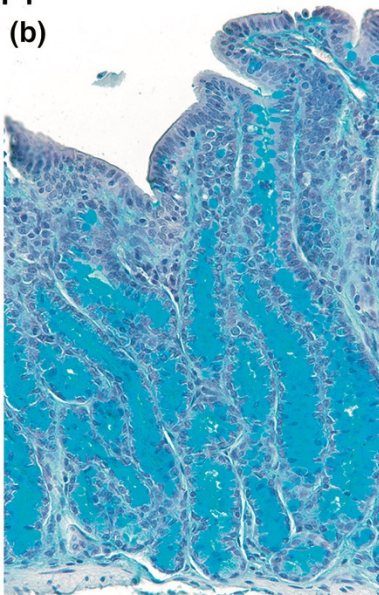
(a')



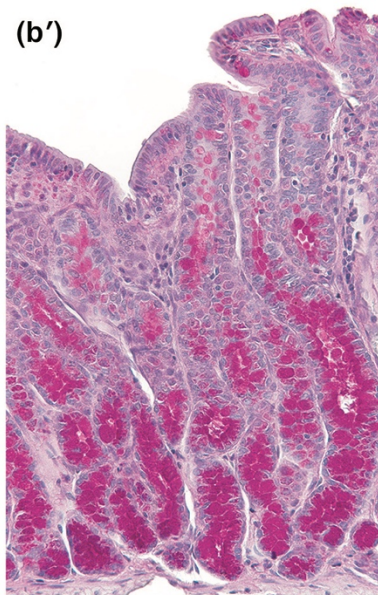
(a'')



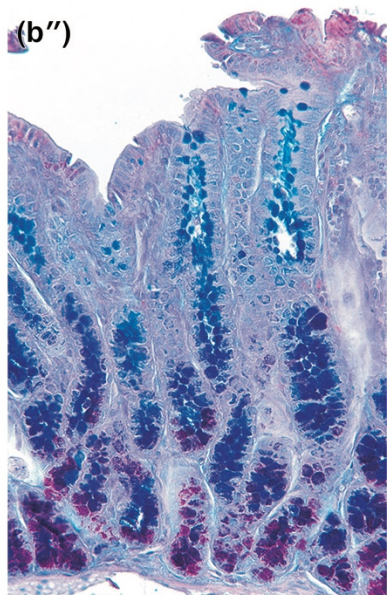
P1
(b)



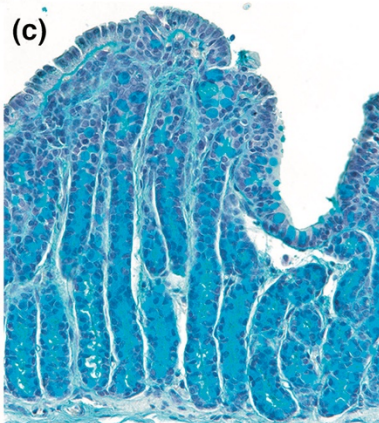
(b')



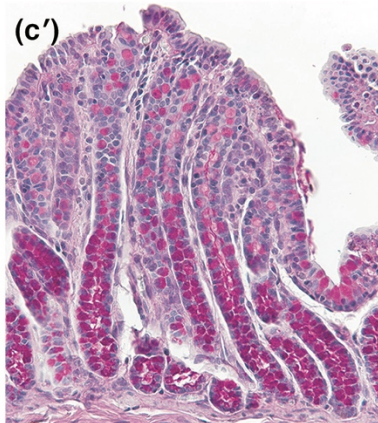
(b'')



P2
(c)



(c')



(c'')

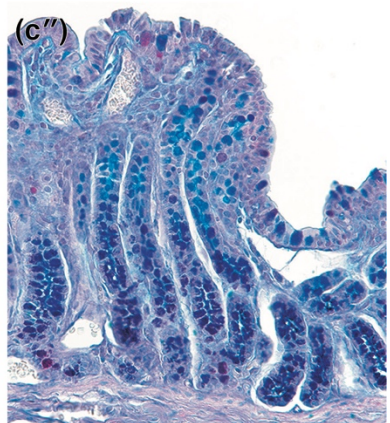
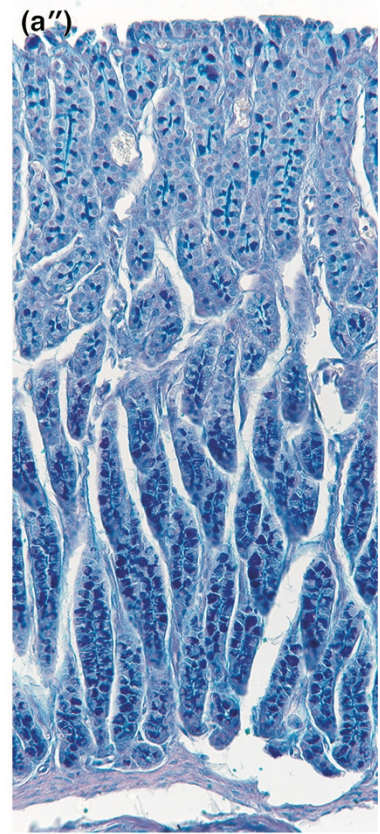
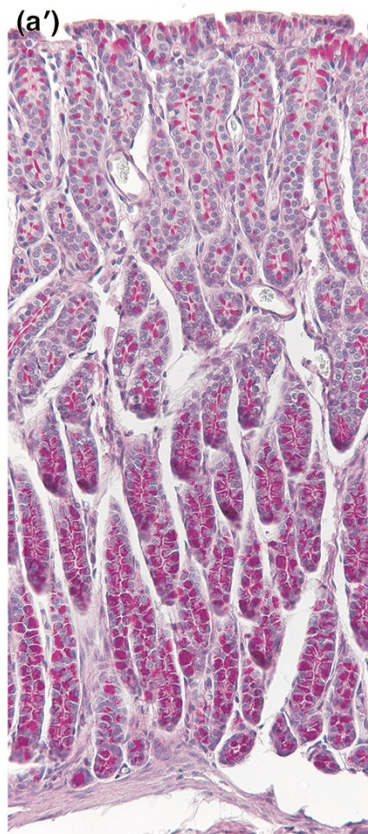
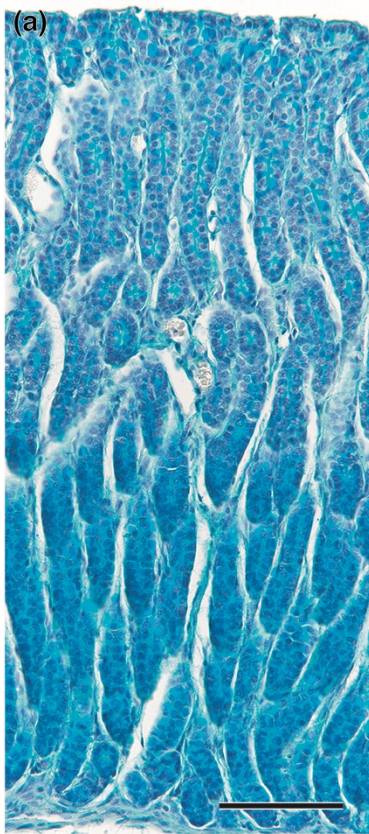
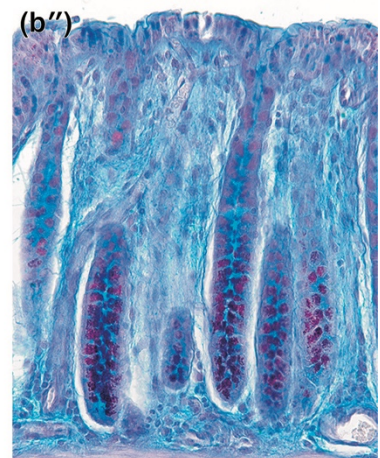
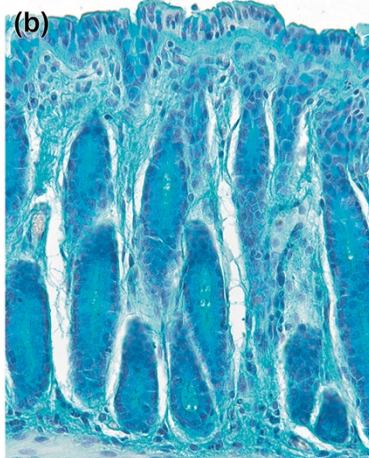


Fig. 7. Cross sections of the fusus coli (a, a' and a''), distal colon (b, b' and b'') and rectum (c, c' and c''), demonstrating the difference in the distribution of mucous cells in each segment. Alcian blue (a, b, and c), periodic acid Schiff (a', b' and c') and combined Alcian blue-periodic acid Schiff staining (a'', b'' and c''). Scale bar, which is applicable to all panels, equals 100 μm .

Fusus coli



Distal colon



Rectum

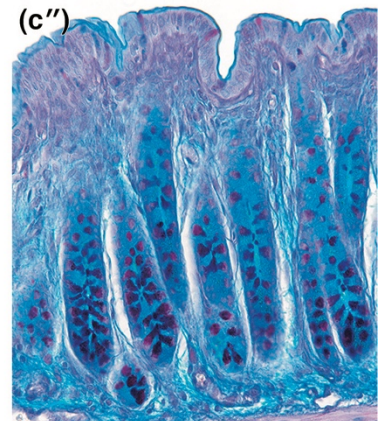
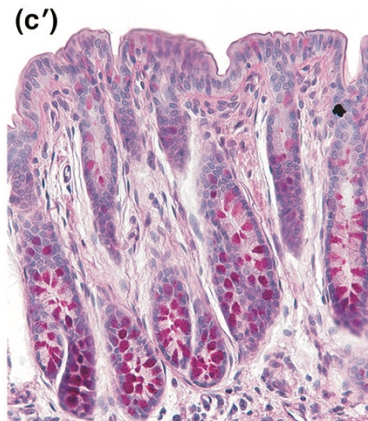
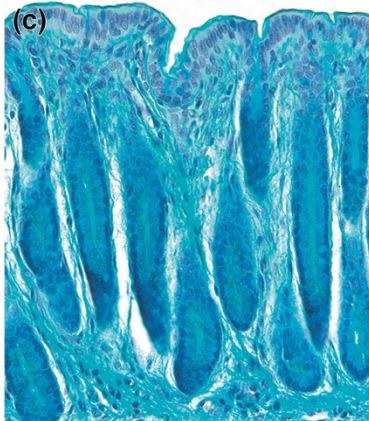


Table 1. Laminar and entire wall thickness (mean \pm SD in μm) of each segment of the rabbit large intestines

Layer	Cecum	P1	P2	Fusus coli	Distal colon	Rectum
Mucosa	136.8 \pm 23.0	406.2 \pm 59.6	307.8 \pm 64.4	627.7 \pm 129.9	313.3 \pm 59.4	279.6 \pm 38.8
Lamina muscularis mucosae	7.5 \pm 2.4	11.5 \pm 6.1	12.3 \pm 4.3	20.8 \pm 16.2	54.2 \pm 9.8	114.6 \pm 20.9
Submucosa	33.1 \pm 13.5	34.5 \pm 17.9	37.1 \pm 28.7	41.5 \pm 23.0	49.2 \pm 12.0	72.3 \pm 31.3
Tunica muscularis, inner circular	63.8 \pm 18.9	132.1 \pm 29.9	255.5 \pm 72.4	203.9 \pm 52.2	157.2 \pm 52.8	350.8 \pm 80.7
Tunica muscularis, outer longitudinal	41.9 \pm 10.4	117.7 \pm 64.2	92.7 \pm 28.5	93.7 \pm 30.8	45.4 \pm 14.2	208.6 \pm 38.5
Serosa	16.6 \pm 6.7	18.0 \pm 7.7	14.9 \pm 9.6	9.6 \pm 9.5	7.1 \pm 4.3	22.4 \pm 8.8
Total	299.7 \pm 32.8	680.6 \pm 117.0	676.7 \pm 123.4	996.9 \pm 168.0	626.4 \pm 115.9	1048.1 \pm 108.4

$n=18$ for each value. For P1 and P2, $n=36$, because their values are calculated across the tenial and haustral parts. For the outer longitudinal layer of the tunica muscularis in P1, $n=24$ and that in P2, $n=19$, because of the occasional absence of the longitudinal muscles. Note that for the serosa in the rectum, the value is of the adventitia.

Table 2. Laminar and entire wall thickness (mean \pm SD in μm) of the tenial and haustral part of P1

Layer	Tenial part	Haustral part
Mucosa	401.8 \pm 51.9	410.6 \pm 67.7
Lamina muscularis mucosae	11.7 \pm 6.1	11.3 \pm 6.2
Submucosa	39.5 \pm 21.9	29.5 \pm 11.2
Tunica muscularis, inner circular	143.9 \pm 22.9	120.3 \pm 31.9
Tunica muscularis, outer longitudinal	152.3 \pm 22.9	14.2 \pm 4.3
Serosa	16.0 \pm 4.2	19.9 \pm 9.8
Total	765.1 \pm 92.2	596.1 \pm 67.7

$n=18$ for each value except for the outer longitudinal layer of the tunica muscularis of the haustral part, where $n=6$. This is due to the occasional absence of the longitudinal muscles.

Table 3. Laminar and entire wall thickness (mean \pm SD in μm) of the tenial and haustral part of P2

Layer	Tenial part	Haustral part
Mucosa	333.2 \pm 70.8	282.4 \pm 46.4
Lamina muscularis mucosae	12.3 \pm 3.2	12.4 \pm 5.2
Submucosa	41.3 \pm 13.1	32.9 \pm 38.6
Tunica muscularis, inner circular	219.6 \pm 53.5	291.5 \pm 72.1
Tunica muscularis, outer longitudinal	95.9 \pm 25.3	NA
Serosa	12.5 \pm 4.1	17.4 \pm 12.7
Total	715.0 \pm 132.0	638.4 \pm 104.0

$n=18$ for each value except for the outer longitudinal layer of the tunica muscularis in the haustral part, where the longitudinal muscles were observed at only 1 site (33.5 μm). Therefore, the value is not applicable (NA) for calculation.