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Morphology of the epithelium of the lower rectum and the anal canal in the adult human

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Abstract The anal canal is an important body part clinically. However, there is no agreement about the epithelium of the anal canal, the anal transitional zone (ATZ) epithelium in particular. The aim of this study is to clarify the structure of the epithelium of the human lower rectum and anal canal. Intact rectum and anus obtained from patients who underwent surgery for rectal carcinoma were examined by light and scanning electron microscopy (LM and SEM). By LM, three types of epithelium were observed in the anal canal: simple columnar epithelium, stratified squamous epithelium, and stratified columnar epithelium. The lower rectum was composed of simple columnar epithelium. SEM findings showed stratified squamous epithelium that consisted of squamous cells with microridges, changing to simple columnar epithelium consisting of columnar cells with short microvilli at the anorectal line. LM and SEM observations in a one-to-one ratio revealed that the area of stratified columnar epithelium based on LM corresponded to the anal crypt and sinus. In conclusion, the epithelium of the human anal canal was fundamentally composed of simple columnar epithelium and stratified squamous epithelium. We found no evidence of the ATZ.

Key words Anal canal · Anal transitional zone · Defecatory function · Scanning electron microscopy · Stratified columnar epithelium

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Introduction

Proctocolectomy with ileal pouch–anal anastomosis (IPAA) has been regarded as a surgical treatment for patients with familial adenomatous polyposis and ulcerative colitis that can avoid permanent colostomy and preserve a better quality of life. However, surgery of the anal canal occasionally causes fecal incontinence. We therefore must understand the anatomic and histological features when we treat diseases in the lower rectum and the anal canal. Recently, intersphincteric resection (ISR) has been introduced as an anus-preserving operation for carcinoma in the extremely low rectum and the anal canal.^{1,2} It is notable that malignant tumors in the anal canal have a wide variety of pathological features.^{3–8} In addition, the anal canal is subject to many common diseases, such as internal hemorrhoids and anal fistula.

Thus, the anal canal is important clinically. However, there is no agreement about the structure of the epithelium of the anal canal.^{9–29} Some definitions are used to describe the anal canal, such as the anatomic, surgical, or histological anal canal. Now, one of the most important discussion points is the matter of the existence of the anal transitional zone (ATZ). The ATZ epithelium has been called transitional epithelium, stratified columnar epithelium, or stratified squamous epithelium. Macroscopic and endoscopic observations are not always sufficient for research in the structure of the epithelium.

In the present study, the epithelium of the lower rectum and the anal canal in the adult human was examined by light and scanning electron microscopy. Especially, the part of the anal column and sinuses identical to the ATZ was observed with great interest.

Materials and methods

Intact parts of the rectum and anus were obtained from patients who underwent surgery for rectal carcinoma at the Department of Surgery II, Oita University. Informed

consent was obtained from all patients. Thirteen specimens were obtained from 13 patients.

Light microscopy (LM)

The anal canal containing the lower rectum was longitudinally cut into 5-mm-thick blocks with razor blades. After being fixed in 10% formalin, the blocks were routinely embedded in paraffin. Sections about 5 μm thick were stained with hematoxylin and eosin and examined under a light microscope.

Scanning electron microscopy (SEM)

We adopted the SEM method of deparaffinized blocks after LM observations to compare the two findings from different angles in a one-to-one ratio (Fig. 1). After observations by light microscopy, the remaining paraffin blocks were deparaffinized with xylene, then hydrated and fixed again in Karnovsky's fixative. Tissue blocks were immersed in 2 N NaOH solution for 1 h at room temperature (25°C) to clean the luminal surface. After being rinsed in physiological saline, all blocks were placed in cacodylate-buffered 1% osmium tetroxide, 1% tannic acid solution, and 1% osmium tetroxide for 1 h each. They were then dehydrated through a graded series of ethanols, dried by the *t*-butyl alcohol drying method, coated with gold, and observed under a Hitachi S-800 scanning electron microscope at an acceleration voltage of 15 kV.

In the present study, we defined the anal canal to begin at the anorectal ring and end at the anal verge from the standpoint of surgery (surgical anal canal). At bedside, it was relatively easy to identify these landmarks. We separated the surgical anal canal into three zones: the upper zone (between the anorectal ring and the anorectal line), the middle zone (between the anorectal line and the dentate line; the part of the anal columns and sinuses), and the lower zone (between the dentate line and the anal verge) (Fig. 2). We recognized that the anal transitional zone was identical to the middle zone, and the suprazonal line and Hermann's line seemed to be identical to the anorectal line, differing from it only in definition.

Results

Light microscopic (LM) findings

Twenty-four slides (1–3 slides per specimen) were observed by light microscopy.

At the lower rectum and the upper zone, the epithelium consisted of simple columnar epithelium and had many crypts. Many huge crypts were also found (Fig. 3).

In the middle zone, corresponding to the anal transitional zone, the epithelium usually consisted of stratified squamous epithelium. This zone was sometimes stratified columnar epithelium, which consisted of some layers of

comparatively tall columnar cells (Fig. 4). There were mainly three patterns in the composition of epithelia in the middle zone (Fig. 5). Patterns I and II showed stratified squamous epithelium alone and stratified columnar epithelium alone, respectively. Pattern III was composed of both stratified squamous epithelium and stratified columnar epithelium. Pattern I was observed on 6 of 24 slides, pattern II on 9 slides, and pattern III on 9 slides. The stratified columnar epithelium was often connected to the excretory ducts of the anal glands or located close to the anal glands (Fig. 6). In most cases, the stratified squamous epithelium or the stratified columnar epithelium suddenly changed to simple columnar epithelium on the anorectal line. However, the stratified columnar epithelium merged smoothly into the stratified squamous epithelium (Figs. 5, 6). In some blocks, stratified squamous epithelium or stratified columnar epithelium partly covered the simple columnar epithelium, and in a few blocks, the components of the two types of epithelium were partly intermingled.

The lower part of the dentate line consisted of stratified squamous epithelium with slight keratinization and papillary formation. The anal verge was characterized by the presence of keratinized stratified squamous epithelium with many melanin granules and hairs.

Histochemical findings

As we recognized the existence of stratified columnar epithelium in the middle zone, we tried to stain the tissue with Alcian blue (pH 2.5 and 1.0) to identify acid glycoprotein. Some cells of the epithelium showed a strong positive reaction for Alcian blue at pH 2.5, which finding illustrated that the columnar cells had mucous granules with acid glycoprotein (Fig. 7).

Scanning electron microscopic (SEM) findings

Eleven tissue blocks of eight specimens (1–3 blocks per specimen) were obtained from eight patients.

SEM observations illustrated clearly that the anorectal line was the boundary between simple columnar epithelium and stratified squamous epithelium or stratified columnar epithelium (Figs. 8, 9).

The area of simple columnar epithelium in the upper zone showed a honeycombed appearance with numerous crypts. In addition, there were some domed structures that had many huge crypts around the anorectal ring (Fig. 10).

In the middle zone, stratified squamous epithelium consisted of thin, polygonal cells that had numerous microvilli (see Figs. 8, 9). Some regions of the anal crypts and sinuses were covered not with squamous cells but with columnar cells, constituting stratified columnar epithelium (Figs. 8, 9, 11). The most superficial surface of columnar epithelial cells was inflated and had short microvilli. Then, the stratified columnar epithelium merged smoothly into the stratified squamous epithelium near the borderline around the anal sinuses in the middle zone. However, in some blocks, the stratified columnar epithelium was enlarged

and reached the area of the simple columnar epithelium of the upper zone, with variations in the width of the middle zone between individuals. Both light and scanning electron microscopic observations in a one-to-one ratio revealed that the area of the stratified columnar epithelium based on

light microscopy corresponded with the anal crypt and sinus (see Figs. 1, 6, 9).

It was clearly established by SEM that the lower zone and the anal verge consisted of stratified squamous epithelium.

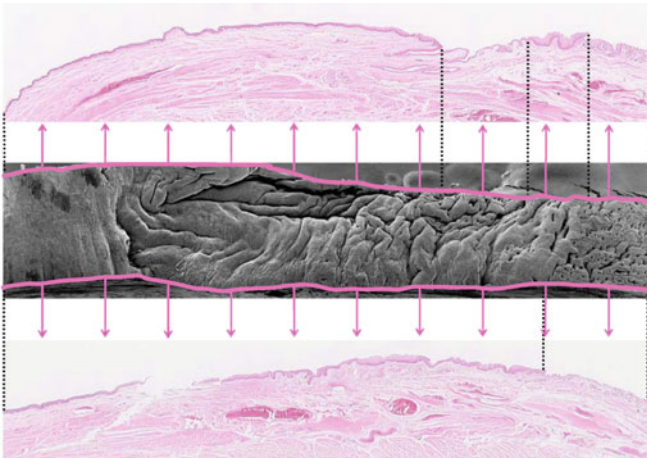


Fig. 1. Light microscopy (LM) and scanning electron microscopic (SEM) findings in a one-to-one ratio

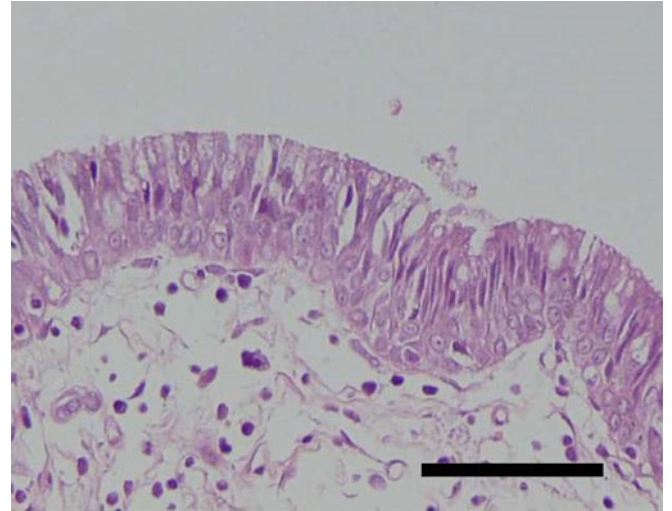


Fig. 4. Stratified columnar epithelium. H&E. Bar 60 µm

Fig. 2. Landmarks of the anal canal

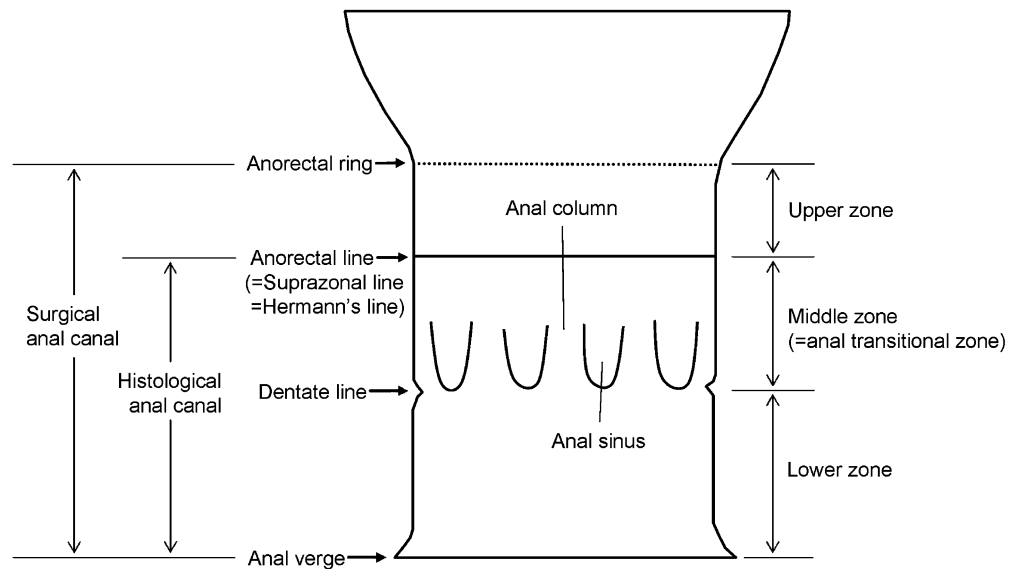


Fig. 3. Huge crypts (arrows). [Hematoxylin and eosin (H&E) staining.] Bar 300 µm

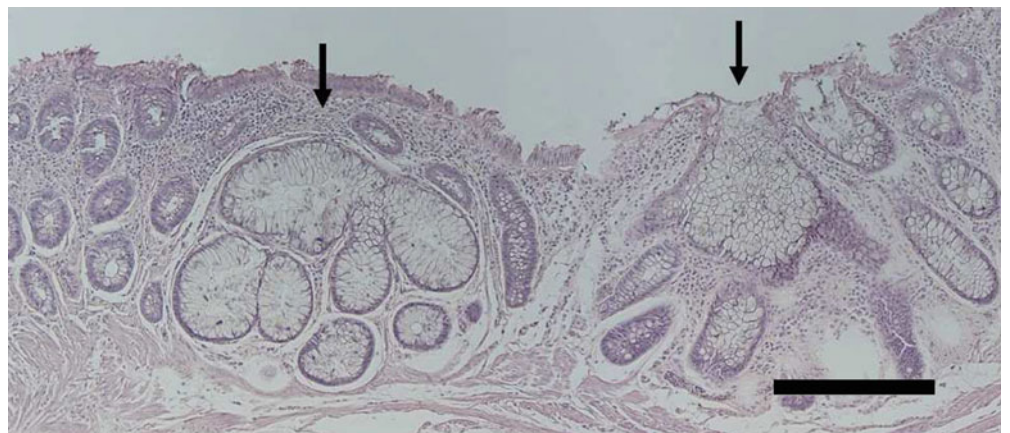


Fig. 5. Three patterns in the composition of epithelia (ep.) in the middle zone. *StSq*, stratified squamous ep.; *StCo*, stratified columnar ep.; *SiCo*, simple columnar ep. *Yellow arrowheads*, transition of the epithelia. H&E. *Bars* 300 μ m

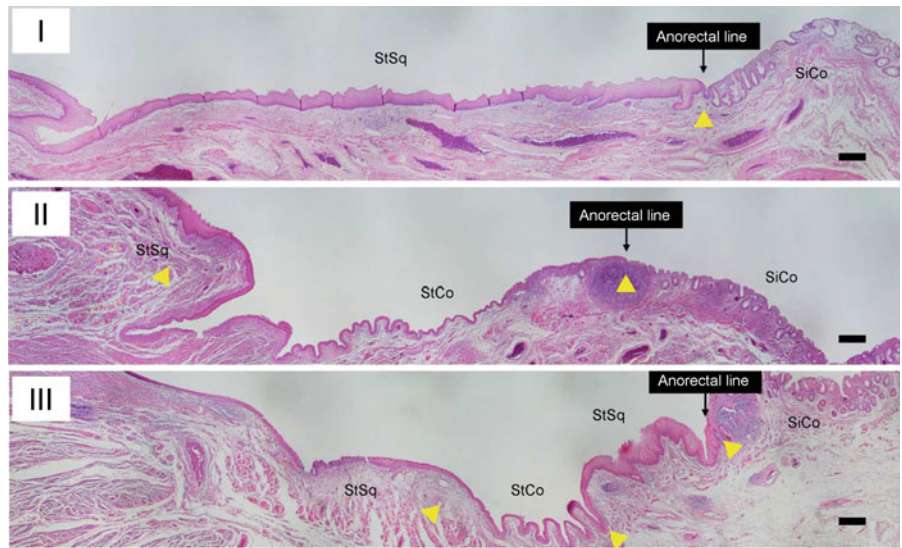


Fig. 6. Relationship among the anal glands (*top*), the ducts of the anal glands (*a*), and the anal sinuses (*b*). H&E. *Bar* 300 μ m

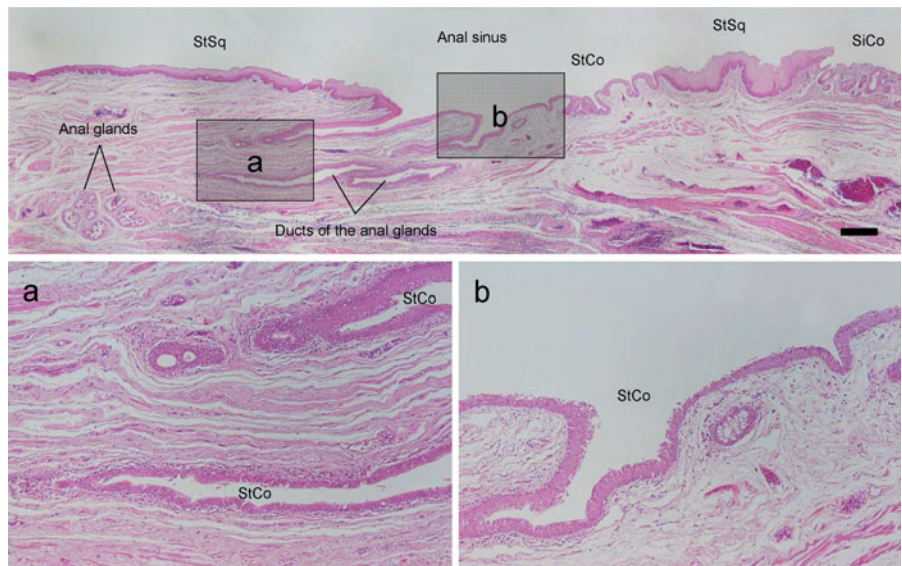


Fig. 7. Histochemical findings of the stratified columnar epithelium (*arrows*). Alcian blue. *Bar* 60 μ m

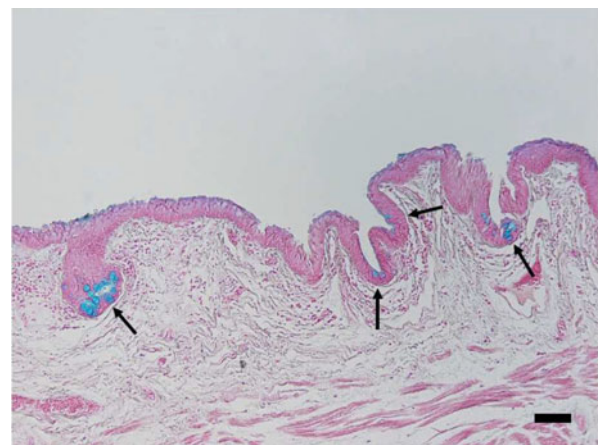
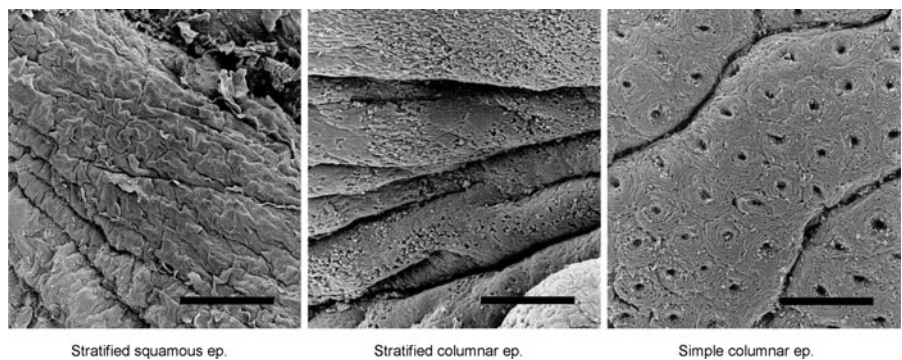


Fig. 8. Types of epithelia (ep.) of the surgical anal canal (SEM). *Bars* 300 μ m



Stratified squamous ep.

Stratified columnar ep.

Simple columnar ep.

Fig. 9. A block containing both the anal sinus and the anal column (SEM). Three types of epithelium were observed. The anorectal line was the boundary between simple columnar epithelium and stratified squamous epithelium (a), and the anal sinus consisted of stratified columnar epithelium (b,c) Bar 1,500 μ m

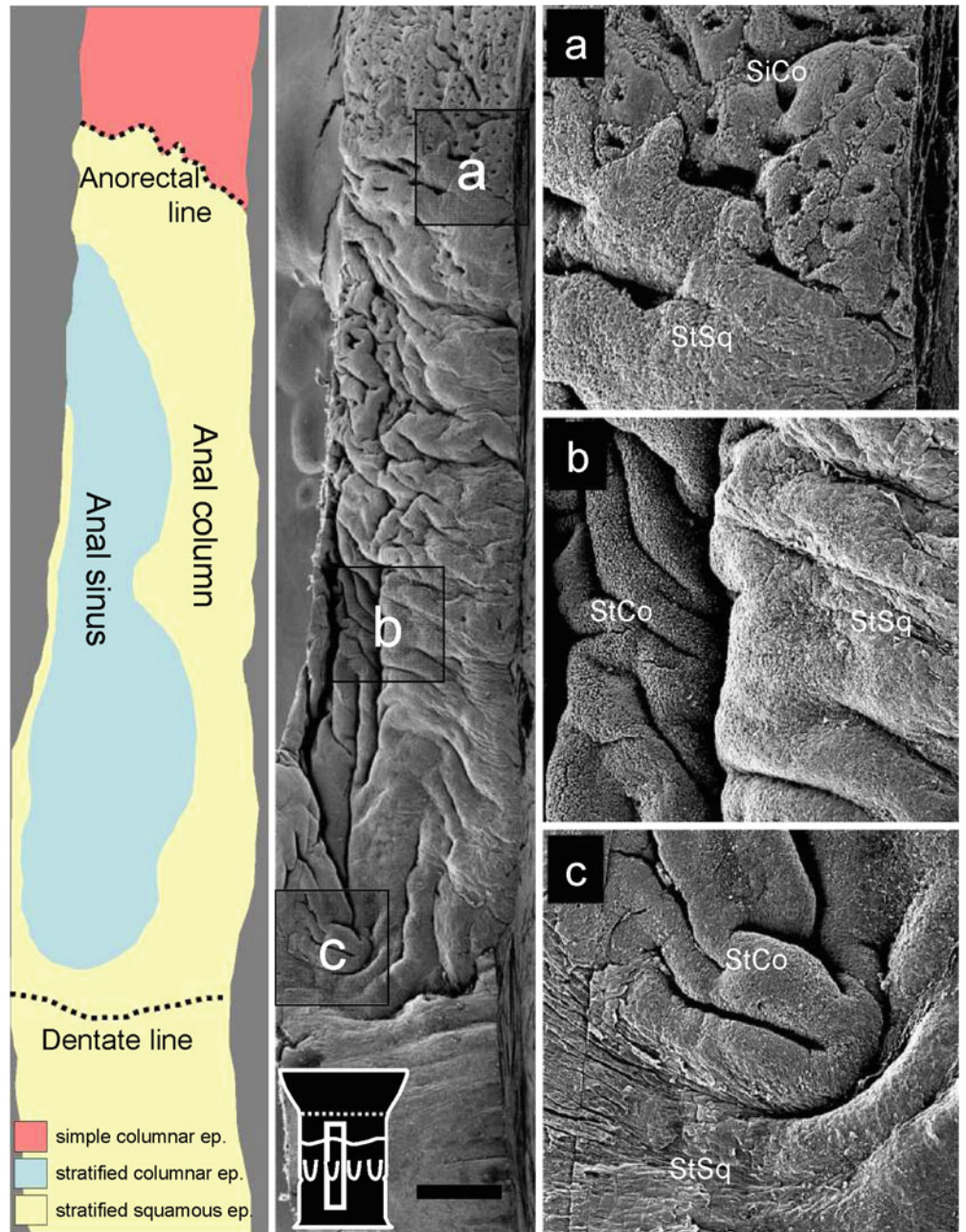


Fig. 10. In the upper zone, there were some domed structures (a: arrow) that had many huge crypts (b) (SEM). Bars 300 μ m

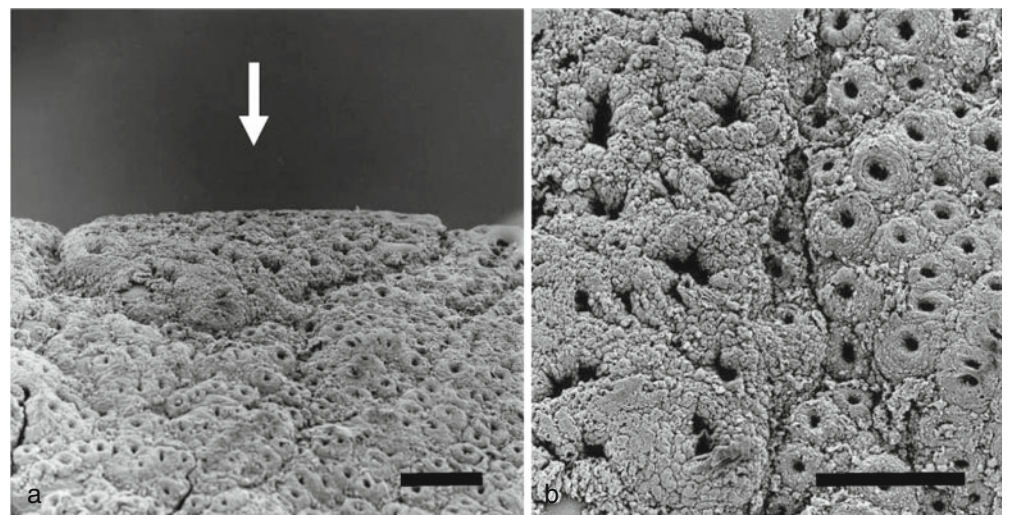


Fig. 11. Stratified columnar epithelium (SEM). Bars 6 μm

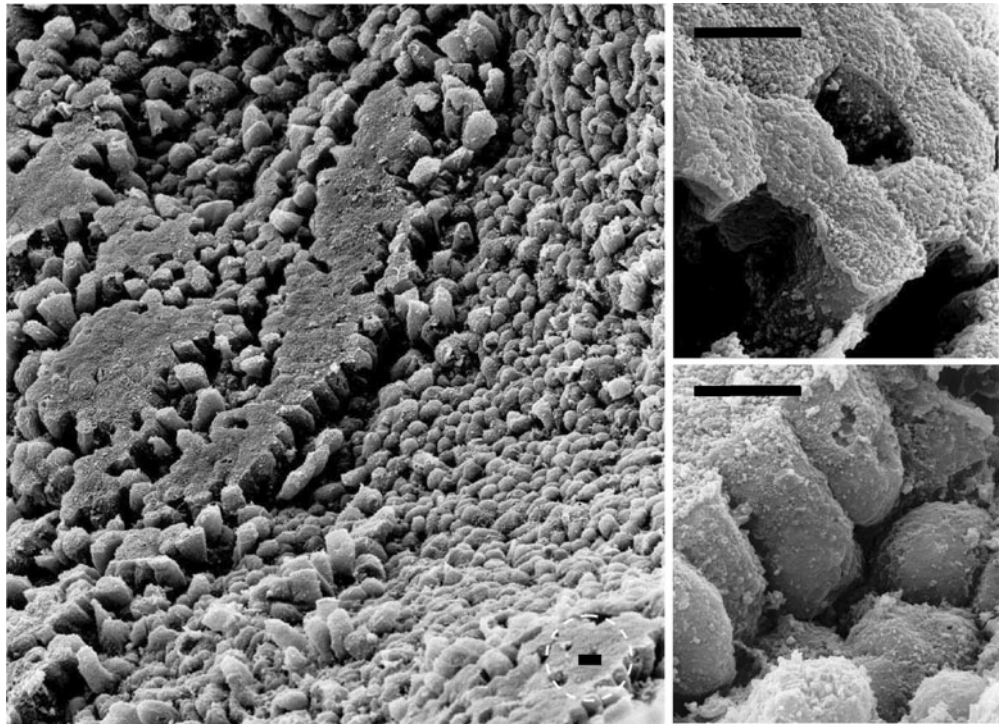


Fig. 12. A simplified schema showing the epithelium of the surgical anal canal based on LM and SEM

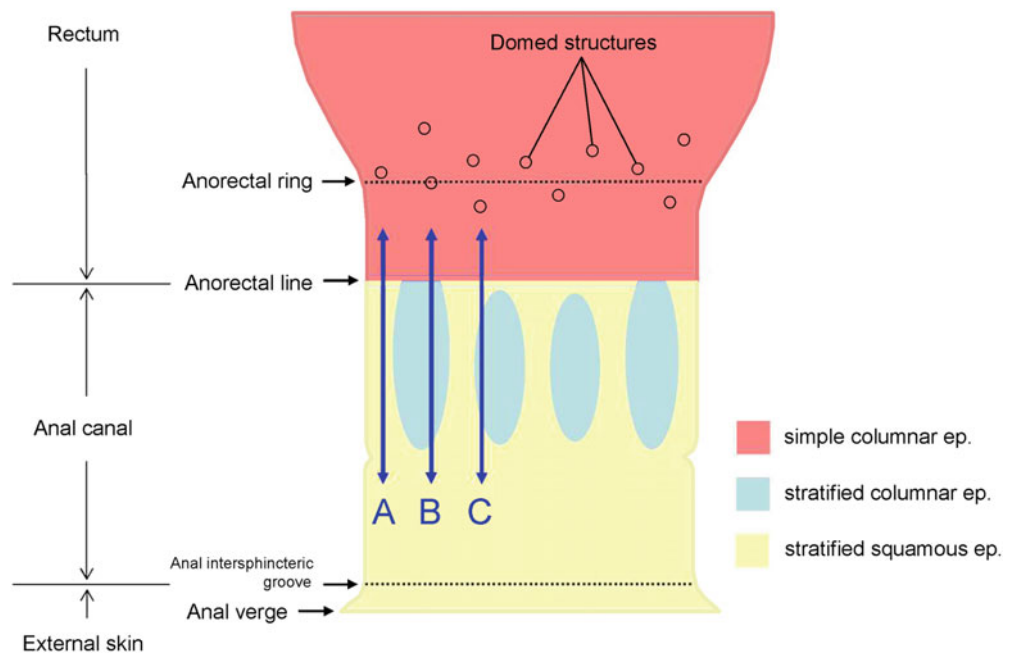
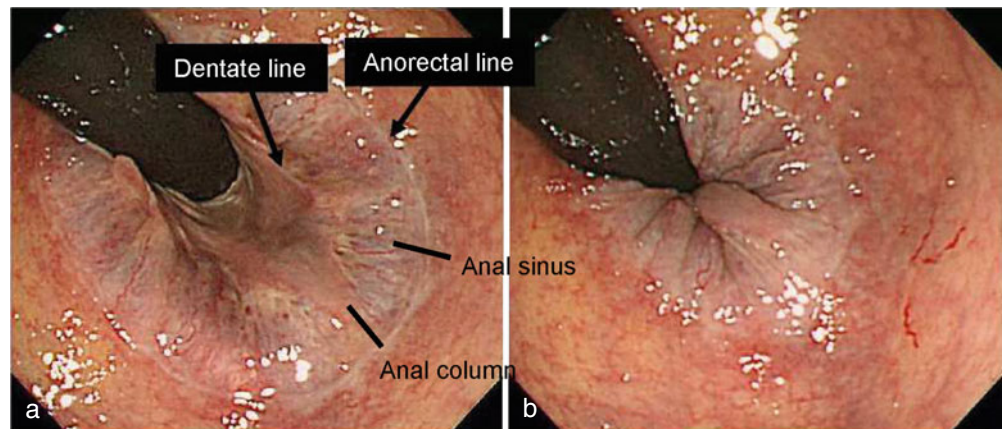


Fig. 13. Endoscopic findings of the anal canal. **a** Inflated rectum filled with feces or gas. **b** Deflated rectum in an empty state



Discussion

So far, there has been no agreement about the structure of the epithelium of the anal canal. Some textbooks described an anal transitional zone or cloacogenic zone between the simple columnar epithelium of the rectal mucosa and the stratified squamous epithelium of the perianal skin,^{3-7,9,16-20,23,24,27-29} but other sources do not refer to this zone.^{10-15,21,22,25,26} The anal transitional zone is usually believed to consist of transitional epithelium,^{7,18,27} stratified columnar or cuboidal epithelium,^{5-7,9,19,23} or various types of epithelia,^{6,9,16,20,27} from only light microscopic or endoscopic findings.

Figure 12 is a simplified schema showing the epithelium of the surgical anal canal, based on LM and SEM images. On the A line, we obtained a longitudinal section through the anal column, and on the B or C line, a section through the anal sinus. Thus, a section sliced on the A line should have shown the light microscopic pattern I in Fig. 5; a section sliced on the B line should have shown pattern II, and a section sliced on the C line should have shown pattern III. As a result, the epithelium of the anal canal has a varied architecture. The epithelium of the surgical anal canal was basically composed of simple columnar epithelium and stratified squamous epithelium, and their borderline was the anorectal line. In addition, the anal crypts and sinuses exceptionally consisted of stratified columnar epithelium. We could detect no special epithelium that could have been called “the anal transitional zone epithelium.”

Stratified columnar epithelium is found only in a few regions, such as the fornix of the conjunctiva, a part of the cavernous part of the male human urethra, and a part of the larger excretory ducts of some glands.^{10-12,15} In the present study, the stratified columnar epithelium enlarging from the anal crypts merged smoothly into the stratified squamous epithelium in the middle zone. Thus, in the anal canal the stratified columnar epithelium appeared to be the medial step from the stratified columnar epithelium of the excretory ducts of the anal glands to the stratified squamous epithelium in the middle zone. In addition, Alcian blue staining revealed that the stratified columnar epithelium had a slight ability to secrete acid mucus.

In general, malignant tumors in the anal canal are rare³⁻⁸ and account for only about 1% of all malignant tumors located in the colon and rectum.⁸ However, adenocarcinoma and squamous cell carcinoma are more frequent.³⁻⁸ Thus, tumors in the anal canal vary in pathological types and patterns, which may arise from the presence of four different types of epithelia in the narrow area, which has a length of only 2.5–5.0 cm.^{3-6,9,16,19,20,23,26,27,30} The four parts are as follows: simple columnar epithelium in the upper zone, stratified squamous epithelium in the middle and lower zones, simple columnar epithelium of the anal glands, and stratified columnar epithelium of the anal sinuses, crypts, and the ducts of the anal glands.

The defecatory functions of the epithelium of the anal canal also vary for each region. Observation from the rectum using an endoscope may suggest that if the lower

rectum were filled with feces or gas, the upper anal canal might slightly open or the epithelium might slide off the anal canal toward the lower rectum (Fig. 13). At this time, feces or gas might come in contact with the epithelium in the middle and lower zone with sensory nerves,³¹ which could be one of the mechanisms of urgency and feces–flatus discrimination. The anal columns and sinuses show a structure resembling an umbrella or an accordion wall (Fig. 13). This structural feature might play a role in smooth expansion of the anal canal without overstretching the epithelium for defecation as well as for continence of feces and gas. The stratified squamous epithelium in this zone appears to be tough, matching the pathway of feces. Then, mucus discharged from not only the anal glands but also the domed structures with huge crypts near the anorectal line might lubricate the surface of the anal canal for smooth defecation. In addition, it also might protect against invasion of antigens. In this way, the epithelium of the anal canal seems to have interesting functional and morphological features for defecation.

The anorectal line was the line at which the simple columnar epithelium in the rectum came to an end. Thus, it seems reasonable that the anorectal line is regarded as the terminus of the large intestine. It is remarkable that the epithelium of the anal canal on the other side of this line is basically composed of one type of epithelium, the stratified squamous epithelium. In addition, this area (which ends exactly at the anal intersphincteric groove) was accompanied by the muscularis mucosae, the circular muscle corresponding to the internal anal sphincter and the longitudinal muscle following those of the rectum. So, we propose that the area that begins at the anorectal line and ends at the anal intersphincteric groove with the internal anal sphincter muscle should be considered and treated as an organ against the rectum or the external skin. When we temporarily defined this organ as the anal canal, it seemed to be simple and easy to recognize (see Fig. 12). Then, according to this definition, the surgical anal canal contains a part of the lower rectum, the anal canal, and the external skin.

The anorectal line is a good landmark when performing an operation on the anal canal, because it is relatively easy to identify by macroscopy and endoscopy. For example, when we perform proctocolectomy with ileal pouch–anal anastomosis (IPAA) for familial adenomatous polyposis or ulcerative colitis, it is better to resect on the anorectal line from the radical and functional point of view. If we resect on the anorectal line, we can theoretically preserve almost all the functions of the epithelium of the anal canal without residual intestinal mucosa. Fichera et al.²⁸ performed a stapled IPAA with preservation of the anal transitional zone (ATZ), the middle zone in the present study, for ulcerative colitis. The result was that preservation of the ATZ offered excellent defecatory function and quality of life and new-onset dysplasia was not noted. Their study revealed that it was valid to preserve the middle zone for defecatory function and may also suggest that the epithelium of this zone differed from the intestinal mucosa.

Conclusions

Light and scanning electron microscopy revealed that the epithelium of the surgical anal canal was fundamentally composed of simple columnar epithelium and stratified squamous epithelium. The borderline was the anorectal line, and we found no evidence of the anal transitional zone.

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