Scanning Electron Microscopic Observations on the Epithelium Lining the Mouse Anorectal Region

By

Takashi NAKANO and Hiroshi MUTO

Department of Anatomy, Aichi Medical University,
Nagakute, Aichi 480-11, Japan

—Received for Publication, June 26, 1987—

Key words: Epithelium, Pectinate line, Rectum, Anal canal, Mouse

Summary: In most cases, the rectal epithelium was abruptly changed into the anal canal epithelium at the pectinate line. In some cases, however, just proximal to the pectinate line there existed the transitional zone between the rectal epithelium and the anal canal one. The epithelium occupying the transitional zone consisted of cuboidal cells with bulging surfaces or flattened cells. It is considered that the epithelium represents various stages which the rectal columnar epithelium transforms into the stratified squamous one. The epithelium lining the mouse anal canal showed an irregular paving-stone-like appearance, which was a typical surface appearance of stratified squamous epithelium, throughout from the anal margin to the pectinate line. The width of the microridges in the distal part of the anal canal was about doubled compared to that in the proximal part. It is suggested that the epithelium in the distal part is keratinized, while that in the proximal part is nonkeratinized.

The junction between the rectal epithelium and the anal canal one, denominating the anorectal junction, represents the comb-like "pectinate line". The pectinate line is the former site of the anal membrane which embryonically divided the ectodermal proctodeum from the entodermal hindgut. It has been pointed out that some types of carcinoma involving the anorectal junction show the wide variation in histopathological pattern, which can not readily be explained on the basis of origin either from the columnar epithelium lining the rectum or from the stratified squamous one covering the anal canal (Grinvalsky and Helwig, 1956; Takano, 1978). Furthermore, the epithelium lining the human anal canal is closely related to the diseases and congenital abnormalities in the anorectal region.

In spite of its embryological and clinical

importance described above, there is still some uncertainty regarding the epithelium involving the anorectal junction and the anal canal. This paper reports some of the findings of the epithelium lining the anorectal region, and is expected to contribute towards further understanding of histology of this region.

Materials and Methods

The mucous membrane in the anorectal region was removed from 30 ddN mice (12 male and 18 female) under a dissecting microscope. Scanning electron microscopic specimens were fixed with glutaraldehyde in phosphate buffer, postfixed with 2% osmium tetroxide, immersed in 2% tannic acid, and stained with 2% osmium tetroxide. They were dehydrated in graded ethanol.

After replacement with isoamyl acetate and drying at critical point with liquid CO₂, they were coated with gold and observed under a Jeol-U3 scanning electron microscope.

Results

The rectal epithelium near the anorectal junction.

The surface of the rectal epithelium is divided by deep furrows to form the anal columns (Fig. 1). At higher magnifications, the surface of the rectal epithelium presents a remarkably geometrical pattern comprising numerous polygonal units, each with a central crypt surrounded by epithelial cells and goblet cells. The goblet cells are arranged radiately about the central crypt (Fig. 2). The crypt is approximately 5-15 μm in diameter, and is surrounded by epithelial cells which appear bent down into the crypt, like a funnel. Many goblet cells are interspersed among the epithelial cells on the crypt wall (Fig. 3).

The junction of the adjacent epithelial cells is easily identified by distinct ridges. Such ridges revealed epithelial cells to be polygonal in surface outlines with irregular pentagonal and hexagonal shapes predominating. The surfaces of the epithelial cells are covered with distinct microvilli, of about 0.1-0.15 μm in diameter (Figs. 4 and 5). Hemispherical projections on the surface may represent the goblet cells bulging with mucus (Fig. 4). The goblet cells, which have presumably discharged their contents, appear as holes 5 μm in diameter (Figs. 2, 4 and 5). Occasionally, septal walls which divide the mucus in the filled goblet cells into various sections are seen in the holes (Figs. 4 and 5).

The anorectal junction

Under the dissecting microscope, the

junction between the rectal epithelium and the anal canal one (the anorectal junction) can be defined, because the rectal epithelium with a dull appearance is abruptly changed into the anal canal epithelium which shows a shiny appearance when viewed in oblique light.

Examination in the SEM reveals that the bases of the anal columns form the anal papillae of various size. The anal papillae give a somewhat serrated or comblike appearance at the anorectal junction, being the pectinate line (Fig. 6). The anal valves are absent. In most cases, at the pectinate line the rectal epithelium is abruptly changed into the anal canal epithelium (Fig. 6). Occasionally, just proximal to the pectinate line there exists the transitional zone between the rectal epithelium and the anal canal one. In some areas of the transitional zone, the superficial cells are cuboidal, of various size and dissected by depressions between the adjacent cells. They bulge out from the surface to a varying degree, presenting a cobblestone appearance (Figs. 7 and 8). In other areas, the superficial cells appear to be relatively flat (Fig. 7). The surfaces of individual cells in the transitional zone have microvilli and microridges, of about 0.1-0.15 μm in width. Various transitional forms between the microvilli and the microridges are observed; e.g., several microvilli lined up closely in a row to form a rosary-like disposition (Fig. 9).

The anal canal epithelium

At low magnification, the anal canal epithelium shows a typical surface appearance of stratified squamous epithelium; i.e. the superficial cells appear to be relatively flat and are arranged in an irregular paving-stone-like appearance with some cells partially overlying those beneath (Fig. 10). Furthermore, superficial cells indicating

various stages of desquamating process are observed.

Higher magnifications reveal that the surface of the anal canal epithelium is somewhat different according to the region of location. In the proximal part of the anal canal, the surfaces of individual superficial cells have microridges of about 0.1-0.2 μm width. In some cells, the microridges with constricted portions are found giving an impression of a fusion of microvilli standing close together (Fig. 11). In other cells, the microridges consist of fine linear ledge. Although the microridges are occasionally arranged parallel to the surface boundaries in the periphery of the cell, there is no specific pattern of arrangement. A few branchings and anastomoses exist (Fig. 12). In the distal part of the anal canal, however, the microridges are approximately 0.3-0.4 μm in width, being about twice the size of those in the proximal part (Figs. 13 and 14). Branchings and anastomoses are more frequent.

As the surface is traced distally, the microridges begin to show a tendency to form adhesion and, therefore, the outlines are not so distinct. Near the anal margin, the surface shows relatively smooth appearance (Fig. 15). However, no typical honeycomb appearance of interconnections of microridges surrounding depressions are observed.

At the anal margin, the anal canal epithelium is changed to the perianal skin with numerous hairs (Fig. 16).

Discussion

Term "anal canal"

For the purpose of this study, the limit of the anal canal is of special importance. Some investigators defined that the human anal canal extended as far as the level of the anorectal ring (Johnson, 1914; Gotoh et al.,

1971; Atsumi, 1978; Ito, 1983) or that of the proximal end of the internal anal sphincter muscle (Fenger, 1979; Fenger and Knoth, 1981). On the other hand, many clinicians regarded the pectinate (dentate) line as the proximal border of the anal canal, since the types of carcinoma derived from the bowel proximal to and distal to the line differ (Hollinshead, 1962). Furthermore, Onitsuka (1970) and Takano (1978) defined the proximal end of the "surgical anal canal" at the level of the anorectal ring, while that of the "anatomical anal canal" at the pectinate line. The pectinate line represents the former site of the anal membrane, which embryonically divided the entodermal hindgut from the ectodermal proctodeum. Furthermore, the two parts of the bowel proximal to and distal to the pectinate line have a different sensory innervation, blood supply and lymphatic drainage.

In this study, the term "anal canal" was defined as the duct extending from the anal margin to the pectinate line, considering not only the anatomical but the embryological findings.

Anorectal junction

As discussed above, in this study the term anal canal is used for the duct extending from the anal margin to the pectinate line; i.e. the pectinate line corresponds to the junction between the rectum and the anal canal. In the human fetus and newborn infant, at the pectinate line there is a sharp transition between the simple columnar epithelium lining the rectum and the stratified squamous one covering the anal canal (Hollinshead, 1962). However, in the adult the issue is more confused. Stonesifer et al. (1960) and Henrich (1980) reported that the simple columnar epithelium was directly changed into the stratified squamous one at or near the pectinate line. On the other

hand, Goligher et al. (1955), Walls (1958) and Onitsuka (1970) reported that the stratified squamous epithelium could extend proximally beyond the pectinate line. Furthermore, many investigators have been reported in man that various types of epithelia, which are neither columnar nor stratified squamous epithelium, exist in the anorectal transitional zone between the simple columnar epithelium and the stratified squamous one, and given the epithelia a variety of names including the "transitional (type) epithelium" (Grinvalsky and Helwig, 1956; Walls, 1958; Klotz et al., 1967; Gotoh et al., 1971; Takano, 1978), "stratified columnar epithelium" (Johnson, 1914; Grinvalsky and Helwig, 1956; Parks, 1956; Walls, 1958; Klotz et al., 1967; Onitsuka, 1970), "cuboidal epithelium" (Onitsuka, 1970; Gotoh et al., 1971; Mitsui et al., 1986), "low stratified polygonal epithelium" (Walls, 1958) and "anal transitional zone epithelium" (Fenger and Knoth, 1981).

The nature of the epithelium occupying the anorectal transitional zone has been obscure. Grinvalsky and Helwig (1956) suggested that the epithelium occupying the transitional zone was a remnant of cloacal entoderm. However, the finding in the fetus and newborn infant, in which the simple columnar epithelium is directly changed into the stratified squamous one (Hollinshead, 1962), makes the suggestion unlikely. On the other hand, Krafka (1940) and Fenger and Knoth (1981) hypothesized that the nature of the epithelium occupying the transitional zone was a modification of the original simple columnar epithelium lining the rectum. In the present study, occasional scanning electron micrographs revealed that just proximal to the pectinate line there existed the transitional zone between the rectal epithelium and the anal canal one. The epithelium occupying the

transitional zone consisted of cuboidal cells with bulging surface or flattened cells. The surface appearances of the epithelium have a close resemblance to those of the squamous metaplastic epithelium in the uterus (Ferenczy and Richart, 1973; Williams et al., 1973) and of the "intermediate epithelium" in the nasopharynx and epiglottis (Nakano, 1986; Nakano and Muto, 1987). The intermediate epithelium showed the various stages of the epithelium transforming from columnar to squamous epithelium (Nakano, 1986; Nakano and Muto, 1987). It is considered that the epithelium occupying the anorectal transitional zone represents various stages which the rectal columnar epithelium transforms into the stratified squamous one. If this is so, then the wide variation in histopathological pattern of carcinoma involving the anorectal junction can be explained.

Epithelium lining the anal canal

It has been reported that the human anal canal is lined with the stratified squamous epithelium throughout from the anal margin to the pectinate line (Johnson, 1914; Goligher et al., 1955; Grinvalsky and Helwig, 1956; Parks, 1956; Walls, 1958; Stonesifer et al., 1960; Onitsuka, 1970; Gotoh, 1971; Moore, 1977; Takano, 1978; Henrich, 1980). On the other hand, Sumida (1962) claimed that the transitional epithelium covered the region between the Hilton's white line and the pectinate line. The Hilton's white line is the linear interval between the external and internal sphincter muscles. Dunphy (1948) described that the stratified squamous epithelium was changed to the transitional epithelium at about the middle point of the anal canal, corresponding the point of the Hilton's white line; i.e. the distal half of the anal canal was lined with the stratified squamous

epithelium, while the proximal half with the transitional one. Fenger and Knoth (1981) reported that the epithelium lining the anal canal near the pectinate line consisted of columnar or cuboidal cells arranged in four to nine layers. Further, Ito (1983) described in his textbook on human histology that the anal canal was partially covered by the stratified squamous epithelium and the columnar one. In this study, the mouse anal canal epithelium showed a typical surface appearance of stratified squamous epithelium throughout from the anal margin to the pectinate line.

The stratified squamous epithelium lining the human anal canal shows the concomitant presence of features denoting similarities to, and differences from, the epidermis, and has assigned a variety of names such as the "anoderm" (Gorsch, 1955; Takano, 1978), "modified skin" (Goligher et al., 1955), "epidermis of the transitional skin" (Baba, 1968) and "specialized anal canal skin" (Henrich, 1980).

It has been the subject of investigation, whether the anal canal epithelium is keratinized or not. Henrich (1980), Fenger and Knoth (1981) and Fujita and Fujita (1986) described in man that the epithelium was nonkeratinized. According to Stonesifer et al. (1960), the epithelium had either a thin cornified layer or none at all. Furthermore, in man (Johnson, 1914) and rhesus monkey (Jit, 1974) it was reported that the distal part of the anal canal was keratinized but the proximal part was not. On the other hand, Walls (1958) and Klotz et al. (1967) mentioned that the human anal canal epithelium was keratinized up to the level of the anal valves. However, it is difficult to conclude whether an epithelium is keratinized or not by use of light microscope only. There is no doubt about the presence of keratinization in normal epidermis. At transmission electron microscopic level, Brody (1959, 1960) observed the "keratin

pattern" with less opaque filaments embedded in an opaque interfilamentous substance in the cornified layer of human and guinea pig epidermis. Baba (1968) observed the keratin pattern in the human anal canal epithelium, but his study was restricted within the distal part of the canal. Furthermore, in most areas of the mucosal epithelium showing less advanced keratinization, the keratin pattern was not observed.

Nakano (1986), one of the present authors, reported at first that the width of intercellular processes and microridges in the cornified layer of the keratinized epithelium was about doubled compared to that in the underlying layers and in the nonkeratinized epithelium, and suggested that the intercellular processes in the keratinized epithelium increased in size, as a result of cell membrane distortion associated with keratinization, to form the tightly applied intercellular interdigitations as the cells moved towards the cornified layer. That is, the difference in width of microridges between keratinized and nonkeratinized epithelia was regarded as an important marker of keratinization. In this study, the width of the microridges in the distal part of the anal canal was about doubled compared to that in the proximal part. It is considered that the epithelium in the distal part of the mouse anal canal is keratinized, while that in the proximal part is nonkeratinized.

It is well known that the surface of the weakly keratinized epithelium tends to show clear microridges, whereas the highly keratinized epithelium does not reveal clear microridges but form a relatively smooth surface; i.e. microridges disappear when the keratinization exceeds a certain degree (Takagi, 1977; Iwasaki et al., 1983; Iwasaki and Miyata, 1985a; b; Iwasaki and Sakata, 1985; Nakano and Muto, 1986; Iwasaki et al., 1987). In this study, as the surface of the anal canal epithelium was

traced distally the microridges became indistinct, and near the anal margin the surface showed relatively smooth appearance instead of clear microridges. Considered from these findings, it is suggested that the epithelium near the anal margin represents more advanced keratinization than in other areas. The cause of the difference in the degree of keratinization has been the matter of discussion. Many investigators e.g. Goetsch (1910) and Parakkal (1967) described in the esophagus that the degree of keratinization was correlated with mechanical stress by diet; i.e. in animals which eat predominantly hard diet the epithelium undergoes more advanced keratinization than that in animals living on soft diet. Furthermore, Hicks (1968) reported that the keratinization in the human urinary bladder epithelium was observed clinically to be associated with irritation by calculi.

As a result of this study, it is reasonable to assume that the anal canal epithelium near the anal margin is subjected to considerable stress in evacuation and is, therefore, more highly keratinized than that in the other areas of the canal. However, there is no gainsaying the possibility that the topographical difference of keratinization is involved in genetic factor.

Acknowledgement

The authors should like to express thanks to Prof. Dr. I. Yoshioka for revising and advising.

References

- 1) Atsumi, K.: Colon sigmoideum, rectum, analis. B. Rectum and anal canal; Fukuda, T. and Hashimoto, Y., *Surgical Anatomy*, Vol. 4 (Japanese). pp. 63-88, Igaku-Shoin, Tokyo, 1978.
- 2) Baba, E.: An electronmicroscopic study of the epidermis of the transitional skin in the human anal canal (Japanese with English summary). *J. Jpn. Soc. Colo-Proctol.*, **21**: 3-41, 1968.
- 3) Brody, I.: The keratinization of epidermal cells of normal guinea pig skin as revealed by electron microscopy. *J. Ultrastr. Res.*, **2**: 482-511, 1959.
- 4) Brody, I.: The ultrastructure of the tonofibrils in the keratinization process of normal human epidermis. *J. Ultrastr. Res.*, **4**: 264-267, 1960.
- 5) Dunphy, J.E.: Surgical anatomy of the anal canal. *Arch. Surg.*, **57**: 791-800, 1948.
- 6) Fenger, C.: The anal canal epithelium, a review. *Scand. J. Gastroenterol.*, (Suppl.) **14**: 114-117, 1979.
- 7) Fenger, C. and Knoth, M.: The anal transitional zone. *Ultrastruct. Pathol.*, **2**: 163-173, 1981.
- 8) Ferenczy, A. and Richart, R.M.: Scanning electron microscopy of the cervical transformation zone. *Am. J. Obstet. Gynec.*, **115**: 151-157, 1973.
- 9) Fujita, H. and Fujita, T.: Organs of digestion, H. Large intestine; *Textbook of Histology Part 2.* (Japanese). pp. 133-135, Igaku-Shoin, Tokyo, 1986.
- 10) Goetsch, E.: The structure of the mammalian oesophagus. *Am. J. Anat.*, **10**: 1-40, 1910.
- 11) Goligher, J.G., Leacock, G. and Brossy, J.-J.: The surgical anatomy of the anal canal. *Br. J. Surg.*, **43**: 51-61, 1955.
- 12) Gorsch, R.V.: *Proctologic Anatomy*. Williams & Wilkins, Baltimore, 1955.
- 13) Gotoh, A., Baba, E., Hayashi, J., Onitsuka, A., Higaki, H. and Ogawa, K.: Discussion on the epithelial tissue of the anus (Japanese). *Surgery*, **37**: 387-392, 1971.
- 14) Grinvalsky, H.T. and Helwig, E.B.: Carcinoma of the anorectal junction. I. Histological considerations. *Cancer*, **9**: 480-488, 1956.
- 15) Henrich, M.: Clinical topography of the proctodeum. *Acta Anat.*, **106**: 161-170, 1980.
- 16) Hicks, R.M.: Hyperplasia and cornification of the transitional epithelium in the vitamin A-deficient rat. *J. Ultrastr. Res.*, **22**: 206-230, 1968.
- 17) Hollinshead, W.: Embryology and anatomy of the anal canal and rectum. *Dis. Colon Rectum*, **5**: 18-22, 1962.
- 18) Ito, T.: Pelvis, II. Organs in the pelvis, C. Rectum; *Human Anatomy* (Japanese), pp. 426-433, Nanzando, Tokyo, 1983.
- 19) Iwasaki, S. and Miyata, K.: Studies on the lingual dorsal epithelium of the guinea pig by scanning electron microscopy. *Okajimas Folia*

- Anat. Jpn., **61**: 423-436, 1985a.
- 20) Iwasaki, S. and Miyata, K.: Light and transmission electron microscopic studies on the lingual dorsal epithelium of the musk shrew, *Suncus murinus*. *Okajimas Folia Anat. Jpn.*, **62**: 67-88, 1985b.
 - 21) Iwasaki, S., Miyata, K. and Kobayashi, K.: Comparative studies of the dorsal surface of the tongue in three mammalian species by scanning electron microscopy. *Acta Anat.*, **128**: 140-146, 1987.
 - 22) Iwasaki, S. and Sakata, K.: Scanning electron microscopy of the lingual dorsal surface of the beagle dog. *Okajimas Folia Anat. Jpn.*, **62**: 1-14, 1985.
 - 23) Iwasaki, S., Sakata, K., Mori, H., Ueno, M. and Kobayashi, K.: Ultrastructure of the lingual dorsal surface in *Suncus murinus* (Linne) (Japanese). *Jpn. J. Oral Biol.*, **25**: 471-480, 1983.
 - 24) Jit, I.: Anatomy of the rectum and anal canal of the rhesus monkey (*Macaca mulatta*). *J. Anat.*, **117**: 271-279, 1974.
 - 25) Johnson, F.P.: The development of the rectum in the human embryo. *Am. J. Anat.*, **16**: 1-57, 1914.
 - 26) Klotz, R.G., Pamukcoglu, T. and Souillard, D.H.: Transitional cloacogenic carcinoma of the anal canal. *Cancer*, **20**: 1727-1745, 1967.
 - 27) Krafka, J.: The creeping epithelium of the anal canal. *Am. J. Surg.*, **49**: 42-48, 1940.
 - 28) Mitsui, T., Shimai, K., Yasuda, K., Kato, S., Kubota, K. and Inoue, Y.: *V. Splanchnologia*, 1. Apparatus digestorius, D. Hindgut, 2. Rectum; *Okajima's Anatomy New Edition* (Japanese), pp. 520-523, Kyorin Shoin, Tokyo, 1986.
 - 29) Moore, K.L.: *The Developing Human* (Jpn. ed.), Ishiyaku-Shuppan, Tokyo, 1977.
 - 30) Nakano, T.: Ultrastructural studies of the transitional zone in the nasopharyngeal epithelium, with special reference to the keratinizing process in the mouse. *Acta Anat.*, **127**: 22-47, 1986.
 - 31) Nakano, T. and Muto, H.: The surface structures of the laryngopharyngeal epithelium of the mouse with special reference to the degree of keratinization. *Okajimas Folia Anat. Jpn.*, **63**: 193-208, 1986.
 - 32) Nakano, T. and Muto, H.: The transitional zone in the epithelium lining the mouse epiglottis. *Acta Anat.*, 1987 (in Press).
 - 33) Onitsuka, A.: Anatomy of the anal canal (Japanese). *Surg. Therapy*, **22**: 150-157, 1970.
 - 34) Parakkal, P.F.: An electron microscopic study of esophageal epithelium in the newborn and adult mouse. *Am. J. Anat.*, **121**: 175-196, 1967.
 - 35) Parks, A.G.: The surgical treatment of hemorrhoids. *Br. J. Surg.*, **43**: 337-351, 1956.
 - 36) Stonesifer, G.L., Murphy, G.P. and Lombardo, C.R.: The anatomy of the anorectum. *Am. J. Surg.*, **100**: 666-671, 1960.
 - 37) Sumida, Y.: Regional anatomy of the anorectum (Japanese). *Surg. Therapy*, **6**: 543-552, 1962.
 - 38) Takagi, T.: Surface structure of the oral mucosa. With special reference to the micro-ridge (Japanese). *Dent. J. Iwate Med. Univ.*, **2**: 125-135, 1977.
 - 39) Takano, M.: Term of the anal canal and its measurement. *J. Jpn. Soc. Colo-Proctol.*, **31**: 226-229, 1978.
 - 40) Walls, E.W.: Observations on the microscopic anatomy of the human anal canal. *Br. J. Surg.*, **45**: 504-512, 1958.
 - 41) Williams, A.E., Jordan, J.A., Allen, J.M. and Murphy, J.F.: The surface ultrastructure of normal and metaplastic cervical epithelia and of carcinoma in situ. *Cancer Res.*, **33**: 504-513, 1973.

Explanations of Figures

Plate I

- Fig. 1. The rectal epithelium. The surface is divided by deep furrows (arrows) to form the anal columns. arrowheads = crypts. X100.
- Fig. 2. Higher magnification to show the geometrical pattern of the rectal epithelium. The goblet cells (arrows) are arranged radiately about the central crypt (arrowhead). X1,000.

Plate II

- Fig. 3. Higher magnification to show a crypt (arrowhead). The wall of the crypt bents down like a funnel. Many goblet cells (arrows) are interspersed among the epithelial cells. X1,800.
- Fig. 4. Higher magnification of Fig. 1. The ridges between adjacent epithelial cells (E) reveal the cells to be polygonal in surface outlines (arrowheads). Hemispherical projection, which may represent the goblet cell bulging with mucus, is seen (small arrow). Other goblet cells appear as holes (large arrows). X3,000.
- Fig. 5. Higher magnification of Fig. 4 to show a goblet cell. Septal walls which divide the mucus are seen (arrows). The epithelial cell (E) is covered with distinct microvilli. X10,000.

Plate III

Fig. 6. The anorectal junction. The rectal epithelium (RE) is abruptly changed into the anal canal epithelium (AC) at the pectinate line (arrowheads). P = anal papillae. X100.

Fig. 7. The transitional zone between the rectal epithelium (RE) and the anal canal epithelium (AC). In some areas of the transitional zone, the superficial cells are cuboidal and present a cobblestone appearance (A). In other areas, the cells appear to be relatively flat (B). arrowheads = pectinate line. X1,000.

Plate IV

- Fig. 8. Higher magnification of Fig. 7 to show the transitional zone. The superficial cells bulge out from the surface. AC = anal canal epithelium. X3,000.
- Fig. 9. The surfaces of individual cells in the transitional zone. Various transitional forms between microvilli and microridges are seen (arrows). X10,000.

Plate V

- Fig. 10. Scanning electron micrograph showing the surface of the anal canal epithelium. The surface shows an irregular paving-stone-like appearance with some cells partially overlying those beneath. $\times 1,000$.
- Fig. 11. Higher magnification of the proximal part of the anal canal. Microridges with constricted portions are observed (arrows). $\times 10,000$.
- Fig. 12. Higher magnification of the proximal part of the anal canal. Some microridges (arrows) are arranged parallel to the boundaries in the periphery of the cell (arrowheads). $\times 10,000$.

Plate VI

- Fig. 13. The surface of the anal canal epithelium. The width of the microridges in the proximal part of the anal canal (A) is thinner than that in the distal part (B). X3,000.
- Fig. 14. Higher magnification to show the difference of the width of the microridges. The microridges (small arrows) in the proximal part of the anal canal (A) is about half the size of that (large arrows) in the distal part (B). X10,000.
- Fig. 15. The epithelium near the anal margin. The surface shows relatively smooth appearance (arrows). X3,000.
- Fig. 16. Scanning electron micrograph showing the anal margin. The anal canal epithelium (AC) is changed to the perianal skin with numerous hairs (PS). X100.