Developmental Changes in the Ampulla Coli of the Afghan Pika (*Ochotona rufescens rufescens*)

By

Masamichi KUROHMARU and Katuya ZYO

Department of Anatomy, Hyogo College of Medicine,
Nishinomiya, Hyogo 663, Japan

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Summary: The morphological changes in the pika ampulla coli, especially the net-arranged folds, from the late fetal stage to the adult stage were examined by the naked eye, light microscopy and scanning electron microscopy.

At 25 days of gestation, the ampulla coli could already be discerned from the cecum and the proximal colon by the lack of haustra. In addition to the well-developed muscular coat, the ampulla coli at this stage was provided with a number of short columnar villi. Although the mucosal surface of the ampulla coli was filled with villi until 3 days after birth, a drastic change occurred at 5 days. That is, the villi gathered together at regular intervals and, as a result, the ampulla coli mucosa revealed a net-arranged appearance at 5 days. At 10 days, the villi disappeared completely and net-arranged folds, derived from the gathered villi at 5 days, covered the ampulla coli mucosa. After 15 days, small villus-like protrusions on the lateral side of the folds developed secondarily and gradually grew in height in accordance with age. At the adult stage, the ampulla coli was overspread with net-arranged folds equipped with small villus-like protrusions.

The large intestine of the pika possesses three conspicuous structures, different from that of the rabbit. The first structure is the ileocecal appendix at the ileocecal junction (Fig. 1). The development of this peculiar lymphoid apparatus was examined by Yamasaki who reported that the ileocecal appendix might be equivalent to the sacculus rotundus of the rabbit (Yamasaki, 1968). The second structure comprises the cecal digitations of the cecal mucosa. The development of these cecal digitations was reported by us in a previous study (Kurohmaru and Zyo.,

1984). It was considered that the cecal digitations might be equivalent to the spiral fold of the rabbit cecal mucosa. The third structure is the ampulla coli, specifically designated "the constricted portion" (Yamasaki and Takahashi, 1963). The ampulla coli of the pika was found to possess a thick wall and specific mucosal folds (Yamasaki and Takahashi, 1963, Puget et Voisin, 1973; Kurohmaru et al., 1984), although that of the domestic rabbit had a thin wall and a flat mucosal surface. The development of this peculiar portion of the pika has not

previously been investigated.

In the present study, the developmental changes of the ampulla coli mucosa of the pika from the late fetal stage to the adult stage were examined by light microscopy and scanning electron microscopy.

Materials and Methods

Pikas (*Ochotona rufescens rufescens*) were obtained from Central Institute for Experimental Animals and were bred in our laboratory. There were fed on a specially prepared solid diet (Matsuzaki et al., 1980) and water *ad libitum*.

Five adult pikas and some postnates in nursing at various postnatal stages (1, 5, 10, 15 and 20 days after birth) were used in the present study. Pika fetuses at 25 days of gestation were also obtained surgically from pregnant females under ether anesthesia.

The ampulla coli from each individual was washed rapidly in Hanks solution for fixation overnight in 2.5% glutaraldehyde with 0.1 M phosphate buffer. Next day, the materials were washed for 1 hr in the same buffer containing 8% sucrose, postfixed for 1.5 hr in 1% osmium tetroxide and dehydrated in graded ethanol. They were then transferred to isoamyl acetate and dried with liquid CO₂ at the critical point. The dried specimens were coated with gold by sputtering in a vacuum evaporator, and observed under a Hitachi S-800 scanning electron microscope.

Some of the specimens were also washed in Hanks solution and fixed in 10% formalin. After observation of the external figure of the ampulla coli at each stage, the samples were dehydrated in graded ethanol and embedded in paraffin. They were sectioned at 5 μ m, stained with hematoxylin-eosin, alcian blue (AB, pH 2.5)-hematoxylin and/or alcian blue-PAS and observed by light microscopy.

Fig. 1. External view of the cecum and ampulla coli in the adult pika. The scale bar represents 1 cm, and the stippled areas show the lymphoid apparatuses. AV: Appendix vermiformis, DI: distal ileum, ICA: ileocecal appendix, PC: proximal colon (first segment), TP: triangular process.

Results

At the adult stage, the ampulla coli of the pika revealed a curved external figure like a stomach and could be definitely discerned from the cecum at its upper side and from the proximal colon at its lower side by the lack of haustra (Fig. 1). The ampulla coli mucosa was covered with net-arranged folds (Fig. 2). In the lateral and basal parts of

the net-arranged folds, small villus-like protrusions project into the lumen in large numbers (Fig. 4). Fig. 2 shows the mucosa in a relaxed condition, while Fig. 3 shows it in a contracted condition. In Fig. 3, small villus-like protrusions are not seen from the dorsal side. The white arrows in Fig. 3 indicate a curved fold, equivalent to the net-arranged fold in Fig. 2. In light micrographs (Fig. 6), the small villus-like protrusions were found to possess AB-positive granules in the upper cytoplasm. In addition, the muscular coat, especially the inner circular muscle, was eminently developed and well-developed muscularis mucosa was also recognized in the interior of the net-arranged folds (Figs. 5 and 6). The contractility of the pika ampulla coli is thus due to these developed muscular layers.

Development of the pika ampulla coli

(1) Macroscopic observations

At 25 days of gestation (gestation period: about 27 days), the ampulla coli of the pika could be already distinguished as a curved stomach-like structure without haustra, different from the cecum and the proximal colon. After birth, the ampulla coli gradually developed in accordance with age. Until 10 days after birth, both the cecum and the ampulla coli possessed scarce and milkwhite contents and revealed a transparent appearance in external view. The pikas might thus ingest only mother's milk during this period, although it has been reported that young pikas could begin to eat at 8 days (Puget, 1973). At 15 days after birth, both the cecum and the ampulla coli were conspicuously developed as compared to those at 10 days. In addition, these portions possessed voluminous and mucous contents and revealed a dark-green appearance in external view, similar to the adult stage. Moreover, the young pikas actually moved after 10 days. There was little doubt therefore that the pikas ate a solid diet to a large degree

at this stage. After 20 days, both the cecum and the ampulla coli became more eminently developed in their volume.

(2) Microscopic observations

At 25 days of gestation, the morphology of the ampulla coli mucosa differed conspicuously from that at the adult stage, although the muscular coat revealed a well-developed appearance similar to the adult stage. That is, the mucosal surface of the ampulla coli at this stage was filled with short columnar villi, in place of the net-arranged folds at the adult stage (Figs. 7 and 8).

At 1 day after birth, the ampulla coli mucosa was also covered with a number of short columnar villi. No intestinal glands were present at the bases of the villi. A well-developed muscular coat was recognized as well as that at 25 days of gestation. Goblet cells were not observed at any period from the late fetal stage to the adult stage (Figs. 9 and 10).

At 3 days after birth, the villous shape changed from columnar to flat leaf-like. Although the light microscopic characteristics were generally similar to the previous stage, shallow intestinal glands were found at the bases of the villi (Figs. 11 and 12).

At 5 days, a drastic change occurred in the mucosal surface. Flat leaf-like villi gathered together at regular intervals and some of them fused with each other. As a result, the ampulla coli mucosa took on a net-arranged appearance, although each villus retained its independence. Intestinal glands developed deeper and the lamina propria of the villi contained some smooth muscle fibers (Figs. 13–15).

At 10 days, the villi vanished from the mucosal surface and the ampulla coli mucosa formed obvious net-arranged folds. A number of sulci ran parallel to one another on the surface of the net-arranged folds. Although the lateral side of the folds

had a concavo-convex appearance with several intestinal glands, small villus-like protrusions, as present at the adult stage, remained underdeveloped at this stage. In light micrographs, well-developed muscularis mucosae were seen in the lamina propria of the folds and were connected with each other (Figs. 16 and 17).

At 15 days, many openings of intestinal glands appeared on the surface of the net-arranged folds. Small villus-like protrusions gradually developed to form a round shape on the lateral side of the folds. AB-positive granules were clearly observed in the upper cytoplasm of the epithelial cells of these small villus-like protrusions (Figs. 18 and 19).

At 20 days, the small villus-like protrusions grew in height to assume a short columnar shape. The surface morphology of the ampulla coli mucosa became similar to that of the adult to a large degree (Figs. 20

and 21).

Summarizing the above findings, the net-arranged fold of the ampulla coli mucosa at the adult stage was derived from the villi at the early postnatal stage. That is, the villi gathered regularly together at 5 days after birth to form net-arranged folds without small villus-like protrusions at 10 days. After 15 days, small villus-like protrusions developed secondarily on the lateral and basal sides of the folds to assume a short columnar shape. As a result, the peculiar structure of the ampulla coli mucosa was virtually accomplished at 20 days (Fig. 22).

Discussion

The ampulla coli mucosa of the pika is covered with villi during the early suckling period, like the pika cecal mucosa during the same period (Kurohmaru and Zyo, 1984). These villi in the large intestine appear to

Fig. 22. Postnatal development of the ampulla coli mucosa in the pika (dorsal view). Until 3 days after birth, the ampulla coli mucosa is covered with flat leaf-like or columnar villi. These villi gather together regularly at 5 days, small protuberances gradually develop on the lateral and basal parts of the folds and form small villus-like protrusions at the adult stage.

contribute to an enlargement of the surface area for the purpose of absorbing the mother's milk more efficiently, similar to those of other mammals (Hilton, 1902; Helander, 1973; Ono, 1980). Generally speaking, they tend to decrease gradually in height and vanish at about 5 days after birth, whereas the villi in the pika ampulla coli do not diminish and contribute to the formation of net-arranged folds after 5 days.

As an example of a similar mode of development, the small intestine mucosa of the field vole can be cited. That is to say, the columnar villi at the early suckling period of the field vole grow wider to fuse gradually from 10 days to 20 days and to form net-arranged folds in the duodenum and wavy folds in the ileum at the adult stage (Kurohmaru et al., 1983). Thus, villi at the early postnatal stage sometimes possess a nature whereby they can secondarily change and contribute to the formation of a different structure.

The net-arranged folds of the field vole duodenum are much smaller in size than those of the pika ampulla coli and are provided with neither protrusions on their lateral side nor granules in their epithelial cells. As regards the formation of the small net-arranged folds in the field vole duodenum, flat villi fuse with each other at their narrower faces. On the other hand, flat villi gather and fuse with each other at their wider faces in the case of the formation of the large net-arranged folds in the pika ampulla coli.

The small villus-like protrusions on the lateral and basal sides of the pika net-arranged fold display a similar shape to villi, but are much smaller in size than villi. They appear to contribute to absorption of the cecal production, since they develop gradually after 15 days.

Judging from the conspicuous contractility of the ampulla coli, it is suggested that the abrupt digesta flow from the cecum

into the ampulla coli appears in the pika according to movements of the valve between the cecum and the ampulla coli, and as a result the ampulla coli swells very much. In the field vole, the abrupt digesta flow from the stomach into the proximal duodenum also appears to lead to swelling of the duodenum according to movements of the pyloric sphincter. In addition, the net-arranged mucosa may be convenient for enlargement and reduction. It seems likely therefore that the small and large net-arranged folds of the two species are the most adaptable to the contractility of the mucosa in each portion.

Moreover, the pika ampulla coli exhibits a curved and swollen appearance just like a stomach. The cecum situated at the upper side of the ampulla coli is much wider than the proximal colon situated at the lower side of the ampulla coli. These facts suggest that the pika ampulla coli may function as a temporary storage receptacle between the cecum and proximal colon, and the contents may flow slowly from the ampulla coli into the proximal colon.

Although such a suggestion can also be adapted to the rabbit ampulla coli, the pika ampulla coli must function more actively than that of the rabbit from a morphological point of view. Likewise, not only the ampulla coli but also the other intestinal tracts of the pika may function more efficiently for absorption than those of the rabbit (Kurohmaru et al., 1984). This may be due to the smaller size of the pika (weight approximately 200 g at the adult stage) than the rabbit (weight more than 4 kg at the adult stage), since smaller mammals may require relatively larger quantities of metabolic energy as compared with their body sizes.

Thus, the ampulla coli of the pika appears to function as a storehouse with a certain absorptive ability. However, further experimental investigations are needed in the

future in order to determine its precise function.

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PLATES

Explanation of Figures

Plate I

Figs. 2–4 Scanning electron micrographs.

- Fig. 2. Ampulla coli mucosa in a relaxed condition at the adult stage. The mucosal surface is covered with net-arranged folds. In the lateral and basal parts of the folds, small villus-like protrusions (arrows) project into the lumen. X60.
- Fig. 3. Ampulla coli mucosa in a contracted condition at the adult stage. The mucosal surface shows a different appearance from that in Fig. 2. In dorsal view, small villus-like protrusions on the lateral side of the curved fold (white arrows) are not seen at all. X70.
- Fig. 4. Higher magnification of small villus-like protrusions. The protrusions are smaller in size, smoother in appearance and sparser in distribution as compared to intestinal villi. X300.

Plate II

Figs. 5, 6, 8 and 10: Light micrographs.

Figs. 7 and 9: Scanning electron micrographs.

Fig. 5. Cross section of the ampulla coli at the adult stage. Hematoxylin-eosin stained.

The lateral side of the fold presents a concavo-convex appearance. Muscularis mucosae are developed very well and connect with each other. X 40.

Fig. 6. Longitudinal section of the ampulla coli at the adult stage. Alcian blue-PAS stained.

The superficial parts of the epithelial cells on the lateral and basal sides of the fold are stained with alcian blue (dark-stained regions), although the epithelial cells at the top of the fold are not stained. X80.

Fig. 7. Ampulla coli at 25 days of gestation. The ampulla coli mucosa is covered with numerous columnar villi. X500.

Fig. 8. Ampulla coli at 25 days of gestation. Alcian blue-hematoxylin stained.

Columnar villi project into the lumen and the muscular coat is very well developed. The upper left region of the photograph is equivalent to the cecum. X 80.

Fig. 9. Ampulla coli at 1 day after birth. As in Fig. 7, the ampulla coli mucosa is covered with columnar villi. X350.

Fig. 10. Ampulla coli at 1 day after birth. Hematoxylin-eosin stained. As in Fig. 8, the ampulla coli possesses numerous columnar villi and a well-developed muscular coat. Neither intestinal glands nor goblet cells are observed at this stage. Goblet cells are not recognized at any stage. X105.

Plate III

Figs. 11, 13 and 15: Scanning electron micrographs.

Figs. 12 and 14: Light micrographs. Hematoxylin-eosin stained.

Fig. 11. Ampulla coli at 3 days after birth. The ampulla coli mucosa is covered with flat leaf-like villi, different from the previous stage. X600.

Fig. 12. Ampulla coli at 3 days after birth. Shallow intestinal glands are clearly seen at the bases of the villi. X105

Fig. 13. Ampulla coli at 5 days after birth. Flat leaf-like villi gather together at regular intervals. Among these gathered villi, regular dents (arrows) are also recognized. The ampulla coli mucosa reveals a net-arranged appearance. X150.

Fig. 14. Ampulla coli at 5 days after birth. Intestinal glands develop deeper and leaf-like villi sometimes appear to fuse with each other. X105.

Fig. 15. Higher magnification of the ampulla coli at 5 days after birth. Flat leaf-like villi are seen to stick together on their wider faces and form a fold-like structure as a whole. X300.

Plate IV

Figs. 16, 18 and 20: Scanning electron micrographs.

Figs. 17, 19 and 21: Light micrographs. Alcian blue-hematoxylin stained.

Fig. 16. Ampulla coli at 10 days after birth. The ampulla coli mucosa reveals net-arranged folds. Numerous sulci run parallel to one another on the surface of the folds, while small villus-like protrusions are not observed on the lateral side of the folds. X100.

Fig. 17. Ampulla coli at 10 days after birth. The lateral side of the net-arranged folds reveals a concavo-convex appearance. The muscularis mucosae of the folds connect with each other. X80.

Fig. 18. Ampulla coli at 15 days after birth. Short protrusions are clearly seen on the lateral side of the net-arranged folds. Numerous openings of intestinal glands are observed on the surface of the folds. X70.

Fig. 19. Ampulla coli at 15 days after birth. On the lateral side of the folds, small villus-like protrusions are developed to a large degree. X80.

Fig. 20. Ampulla coli at 20 days after birth. Net-arranged folds become provided with small villus-like protrusions on the lateral side. The ampulla coli mucosa reveals a similar appearance to the adult stage. X50.

Fig. 21. Ampulla coli at 20 days after birth. The muscular coat appears well-developed. On the lateral side of the folds, small villus-like protrusions exhibit a columnar shape. X40.