Morphological and Histological Studies on the Gastric Mucosa of the Japanese Field Vole, Microtus montebelli montebelli

Masamichi KUROHMARU*, Takao NISHIDA and Koshi MOCHIZUKI

Laboratory of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113

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Abstract. The gastric mucosa of herbivorous field voles was examined with the naked eyes, a light microscope, and a scanning and a transmission electron microscope. Its morphological characteristics were compared with those of omnivorous golden hamsters. The stomach of the hamster consists of two parts, forestomach and glandular stomach, whereas that of the field vole is composed of three parts, esophageal sac, fundic stomach and pyloric stomach. The fundic stomach has a number of gastric glands which open by oval pits on the mucosal smooth surface. The esophageal sac mucosa and the pyloric stomach mucosa are connected with each other on the side of the lesser curvature, both the mucosal membranes being lined with stratified squamous epithelia. The mucosal surface of the esophageal sac has numerous complicated folds, whereas that of the pyloric stomach has a succession of regularly and compactly arranged protrusions. A fimbria, specific to the field vole, is observed between the esophageal sac and the fundic stomach, making a definite distinction of these two parts. The fimbria appears to strengthen the function of the esophageal sac as a fermental vat. The pyloric gland region is separated from the fundic stomach by the pyloric stomach and is located at the short part just before the duodenum. The functional roles of the pyloric stomach and the pyloric gland segment are not clear.

It is well known that the field vole (genus Microtus) is one of the rodents most adapted to the herbivorous food-habit and has the pregastric fermentative digestive system.

In recent years, the field vole has been gradually noticed as a new experimental herbivora [2, 3, 7, 13]. In addition to the large extended cecum resulting from its adaptation to herbivorous habit [4, 14], the animal has three stomachs, esophageal sac, fundic stomach and pyloric stomach.

Although many studies have been accumulated on these three stomachs from the viewpoint of nutritional physiology [7, 10], very few studies have been made from the viewpoint of morphology except for the Microtus pennsylvanicus [4].

In the present investigation, the stomach of the Japanese field vole, Microtus montebelli, was observed with the naked eyes, a light microscope and a scanning and a transmission electron microscope, in order to elucidate its morphological characteristics in more detail. The gross features of the external and internal figures and the surface structures of the stomach in the field vole are compared with those in the golden hamster. The histological characteristics of the stomach of the field vole are also discussed.

* Present address: Department of Anatomy, Hyogo College of Medicine, Mukogawa-cho, Nishinomiya 663.
Materials and Methods

Materials used were composed of nineteen adult field voles (thirteen males and six females) weighing 47 g for male and 32 g for female on an average and four adult male golden hamsters weighing 105 g on an average.

These field voles were derived from the breed stock in Nippon Veterinary and Zootechnical College, bred in our laboratory and fed a diet for the herbivore. Golden hamsters were also bred in our laboratory, fed a diet for mice.

The stomachs from four field voles and two hamsters were excised in toto under ether anesthesia, fixed for a few hours in 2.5% glutaraldehyde with 0.1 M Sörensen phosphate buffer and then cut in half to observe their internal figures.

The stomachs obtained from the other five field voles were cut in half in a similar way, fixed in glutaraldehyde, dehydrated in a graded series of ethanol and embedded in paraffin. These samples were sectioned at 7 μm, stained with hematoxylin-eosin or periodic acid Schiff (PAS) and observed by light microscopy.

From the remaining field voles and hamsters, small segments of their esophagus and stomachs were cut off and were washed rapidly in Hanks solution. These segments were fixed for a few hours in 2.5% glutaraldehyde with 0.1 M Sörensen phosphate buffer, washed overnight in the same buffer containing 8% sucrose, postfixed for 1.5 hr in 1% osmium tetroxide with the same phosphate buffer and then dehydrated with graded ethanol. The samples thus prepared were transferred to isooamyl acetate, dried with liquid CO₂ at critical point, coated with gold by sputtering in a vacuum evaporator, and then observed by a JSM-U3 scanning electron microscope. For transmission electron microscopy, only the materials of field voles were transferred to n-butyl glycidyl ether and were embedded in Epon 812. Then, the thick sections were stained with toluidine blue and were observed by light microscopy. The thin sections were stained with lead citrate and uranyl acetate and were observed by a JEM-100S transmission electron microscope.

Results

I. Gross characteristics

As shown in Fig. 1, the fundamental external feature of the whole stomach of the field vole was largely similar to that of the golden hamster.

The stomach of the golden hamster consisted of two parts, forestomach and glandular stomach, whereas that of the field vole was divided into three parts, esophageal sac, pyloric stomach and fundic stomach. The pyloric stomach was independent of the fundic stomach. Additionally, an isthmus was distinctly observed between the pylorus and the duodenum in the field vole.

The forestomach mucosa of the golden hamster was lined with a keratinized epithelium as seen in the esophagus, distinguishable from the fundic stomach mucosa by a short upheaval (Fig. 2). The smooth surface of the fundic stomach was extended toward the pyloric region without any remarkable changes.

In contrast, in the stomach of field vole, although similarly lined with a keratinized epithelium, the esophageal sac mucosa was connected obviously with the pyloric stomach mucosa on the side of the lesser curvature. Thus the pyloric stomach mucosa was also lined with the keratinized epithelium.

The fundic stomach mucosa of the field vole represented a smooth-surfaced appearance similar to that of the hamster. A characteristic triangular fimbria formed a boundary between the esophageal sac and the fundic stomach, and a short mucosal protrusion demarcated a region between the fundic and the pyloric stomachs.

No fimbria was recognized in the region transforming from the esophageal sac to the pyloric stomach. The mucosal folds of the esophageal sac and pyloric stomach in the field vole were by far rougher than those of the forestomach in the golden hamster.

II. Surface structures

The structures of mucosal surfaces in the esophagus, esophageal sac and pyloric stomach of the field vole were observed by scanning electron microscopy.
The mucosal folds of the esophageal surface ran regularly side by side, perpendicularly to the long axis of the esophagus. At a high magnification, fold-like projections were observed to run longitudinally with constant intervals.

In the esophageal sac, numerous complicated small mucosal folds existed on the large mucosal folds which often disappeared by expansion of the sac (Fig. 3). At a high magnification, fold-like projections were observed also on the surfaces of the small mucosal folds. These projections appeared to be similar to those observed on the esophageal surface but were irregularly arranged (Fig. 6).

The mucosal folds of the pyloric stomach were developed as well as those of the esophageal sac, composed of a succession of regularly compacted folds (Fig. 4). At a higher magnification, the mucosal surface of the pyloric stomach appeared to be almost the same as that of the esophageal sac.

On the other hand, the smooth mucosal surface of the fundic stomach was provided with a great number of oval gastric pits each of which opened independently (Fig. 7).

Epithelial cells surrounding each gastric pit protruded moderately. Although no demarcation between the esophageal sac and the pyloric stomach could be made with the naked eyes, the gradual transition from the esophageal sac mucosa to the pyloric stomach mucosa was observed in the narrow part on the side of the lesser curvature by scanning electron microscopy, appearing to show a demarcation between these two gastric parts.

The keratinized epithelial lining abruptly terminated at the isthmus present in the end of the pyloric stomach, and the region just followed by the duodenum was covered with the smooth mucosal surface which had gastric pits as did the surface of the fundic stomach. This region between the isthmus and the duodenum was 3 or 4 mm in length, probably corresponding to the real pylorus and being distinctly transferred to the duodenal mucosa (Fig. 10). Hence, this region may be called the pyloric gland segment, where a number of microorganisms attached to the mucosal surface. A majority of these microorganisms were spheroidal, different from the rod-shaped bacteria seen on the esophageal sac mucosa (Figs. 11 and 12).

Scanning electron microscopy for the forestomach of the golden hamster revealed that it was lined with a stratified squamous epithelium (Fig. 5) just as observed on the surfaces of the esophageal sac and the pyloric stomach in the field vole (Figs. 3 and 4). The mucosal surface of the forestomach in the golden hamster had regular small protrusions similar to those found on the esophageal sac mucosa in the field vole. The mucosal surface of the glandular stomach in the golden hamster had also oval gastric pits, but the pits were often fused with each other (Fig. 8). The stomach of the golden hamster had neither fimbria nor pyloric stomach nor pyloric gland segment.

III. Histological structures

The mucosal surfaces of the esophagus, esophageal sac and pyloric stomach in the field vole appeared to be somewhat different from each other, on the basis of scanning electron microscopic observation, but histological observation showed that the structures of these three parts were almost identical, because their mucosal membranes were all similarly composed of the keratinized epithelium, lamina propria, muscularis mucosa, submucosa, muscularis externa and
serosa (Fig. 15).

In the stratum basale or stratum spinosum, there were a few layers of epithelial cells containing large and oval nuclei, scarce cytoplasm, a few mitochondria and well-developed intercellular bridges. In the stratum granulosum, several layers of flattened and slender cells were densely packed and these cells had obscure elongated nuclei, some degenerating mitochondria, numerous tonofibrils, small middle electron-dense granules and keratohyalin granules. The keratohyalin granules were high in electron density, variable in size; most of them were oval and some were elongated (Fig. 13). Cells in the stratum corneum were flatly stacked, having neither nuclei nor organelles.

The triangular fimbria in the field vole was lined with a keratinized stratified squamous epithelium which continued from the esophageal sac mucosa (Figs. 9 and 17).

The mucosa of the fundic stomach abounded with gastric glands, and the muscularis externa appeared to be thin (Fig. 16). When examined by light microscopy, however, each gastric gland was observed to consist of three segments, base, neck and surface (Fig. 16).

The surface of the gastric mucosa was covered with surface epithelial cells. These surface mucous cells were columnar, PAS-positive and darkly stained with toluidine blue. The oval nuclei of these cells were located in the basal part and were scarce of chromatin. The free surface of these cells was almost flat, but was studded with some short protrusions. In the cytoplasm, there were several round or oval mitochondria, numerous tonofibrils, small round granules, and low electron-dense materials with amorphous structures (Fig. 19).

The thin portion of the glandular neck was composed of mucous neck cells which were lightly stained with hematoxylin eosin and darkly stained with toluidine blue or PAS. These mucous neck cells appeared to consist of three or four layers. The nuclei were somewhat flattened, scarce of chromatin and located at the basal part of the cell. A great number of oval secretory granules were accumulated at the apical part of the cell. These secretory granules had a lower density and were more uniform in size than zymogenic granules seen in chief cells (Fig. 20).

The basal portion of the gastric gland was filled with a succession of chief cells and parietal cells. The chief cells, darkly stained with hematoxylin-eosin or toluidine blue, were observed more numerously in the basal part. Their nuclei were oval, abounding with chromatin. The whole cytoplasm was occupied by zymogenic granules and well-developed rough endoplasmic reticula. In the supranuclear region, there were a few mitochondria and a Golgi complex.

The zymogenic granules varied in size and density. Smaller granules, which were thought to be immature, appeared frequently around the Golgi complex (Fig. 22). The parietal cells, lightly stained with toluidine blue, were distributed almost uniformly throughout the basal portion. Their nuclei were scarce of chromatin, different from chief cells. A number of round mitochondria, smooth and endoplasmic reticula and underdeveloped intracellular secretory canaliculi were present in the cytoplasm (Fig. 21). In the pyloric gland segment, the mucosal epithelium was composed of a certain type of mucous cells. These mucous cells contained round and low-dense granules in the apical cytoplasm, morphologically different from the surface mucous cells and the mucous neck cells in the fundic stomach. The pyloric glands
were found clearly and continued to the duodenal submucosa. In addition, among numerous mucous cells were frequently encountered special cells which were granulated in their basal part of the cell body, presumably identifiable as gastrin cells (Fig. 14).

Discussion

In the stomach of *Microtus montebelli*, the pyloric gland exists, independent of the gastric gland as reported for *Microtus pennsylvanicus* [4]. However, the pyloric gland area is limitedly smaller in the *Microtus montebelli* than in the *Microtus pennsylvanicus*. Golley [4] called this area "pyloric stomach". In addition, the "esophageal sac" described by Golley [4] includes not only the forestomach but also that pyloric part which has no pyloric glands.

In this paper, however, the terms of "esophageal sac", "fundic stomach" and "pyloric stomach" are applied to respective parts of the stomach in the *Microtus montebelli* as shown in Figs. 1 and 2, based on the morphological differences found in these parts. The short region between the isthmus and the duodenum, possessing the pyloric glands, should be called "pyloric gland segment". On the other hand, the pyloric gland of the *Microtus pennsylvanicus* is located at the pyloric part just before the isthmus. Thus the pyloric gland is distinctly different in location as well as in width between the *Microtus montebelli* and the *Microtus pennsylvanicus*. It is very interesting that these two species, belonging to the same Genus, are different in location of the pyloric gland area.

Light and transmission electron microscopies revealed that the esophageal sac and pyloric stomach of the field vole were lined with the keratinized stratified squamous epithelia which can be also seen in the forestomach of the golden hamster [5, 12]. Although the fundic stomach of the field vole is obviously thicker in width than the glandular stomach of the golden hamster, no distinct differences are observed in the histological structures between the stomachs of these two species [6]. The ultrastructures of four types of cells, which compose the mucous epithelium, as well as of gastric gland of the fundic stomach in the field vole also appear to be similar to those in other species of rodents [1, 8, 9]. However, some differences are found in such a manner that small granules contained in the surface mucous cells are round in the field vole but oval in the mouse [8], and a manner that the intracellular canaliculi of the parietal cells are underdeveloped in the field vole as compared with those in the rat [9].

In comparison of the gross characteristics of the stomach between the field vole and the golden hamster, the most important morphological difference is the presence of a fimbria specific to the field vole. This fimbria makes it possible to distinguish the esophageal sac from the fundic stomach and probably serves for prevention of the back flow of the contents in a way similar to that of the terminal valve located between the omasum and the abomasum in ruminants. It is also suggested that the esophageal sac may serve as a fermental vat more efficiently than the forestomach of the golden hamster, owing to the existence of the fimbria. The esophageal sac of the field vole had more complicated and rougher mucosal folds than had the forestomach of the golden hamster. Such numerous mucosal folds may extend the surface area and contribute to the effective absorption of volatile fatty acids fermented in the sac.

The pyloric stomach and the pyloric gland segment were present specifically in
the stomach of the field vole. Although their exact functional significance is not known at present, it is assumed that the pyloric stomach is little related to the fermentation, judging from its regularly surfaced appearance and its rare movement compared with the esophageal sac, and also from the low concentration of volatile fatty acids in it [10]. In many of the omnivorous rodents possessing the mono-stomach, since the anterior part of the stomach is covered with the keratinized epithelium [15], food may be transported to the anterior part of the stomach (corneus region), the glandular stomach (glandular region), and the duodenum in turn.

On the contrary, in the field vole, food may be moved to the esophageal sac (corneus region), the fundic stomach (glandular region), the pyloric stomach (corneus region), the pyloric gland segment (glandular region), and the duodenum in turn. During this movement, food may be gradually changed to a more and more absorbable type.

Although a variety of forms in stomach has been observed in rodents [15], the more detailed morphological studies are confined to a few popular species, such as rat, mouse and hamster [1, 8, 9, 11, 12]. In order to clarify the functional significance of the pyloric stomach and pyloric gland segment specific to the field vole, the stomachs of those other species of rodents which have similar three gastric parts will be necessary to be examined in detail.

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References

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要　約

ハタネズミの胃粘膜に関する形態学的研究：九郎丸正道・西田隆雄・望月公子（東京大学農学部家畜解剖学教室）— 駆食性実験動物として開発されたハタネズミの胃粘膜を、肉眼、光学顕微鏡、走査型および透過型電子顕微鏡を用いて観察し、雑食性のハムスターのそれを比較検討した。ハムスターの胃が前胃および腺胃の2つの部位からなるのに対し、ハタネズミの胃は食道胃、腺胃および幽門胃の3つの部位からなる。この3部位のうち中間にある腺胃は多数の固有胃腺を含み、その粘膜表面には多くの彎曲形の胃小窩が開口する。一方食道胃および幽門胃は小腸部において連続し、ともに角化重層扁平上皮によっておおわれる。食道胃粘膜表面は多数のヒグ状隆起が複雑に配列して走るが、幽門胃粘膜表面は規則正しい小隆起構造が連続する。食道胃と腺胃の間には、ハタネズミに特有のフィンブリアとよばれる芽様構造物が存在する。このフィンブリアは食道胃の独立性を強め、その発酵槽としての役割を高めるように働くと考えられる。またハタネズミの幽門腺部は腺胃から幽門胃によって隔てられ、十二指腸近位端の幅数 mm の部分に局在する。これら幽門胃および幽門腺部の機能的な意味については明らかでない。

Explanation of Figures

Abbreviations (Figs. 1 and 2)

I: esophageal sac.
III: pyloric stomach.
V: glandular stomach.
ES: esophagus.
FI: fimbria.

II: fundic stomach.
PV: forestomach.
DU: duodenum.
PG: pyloric gland segment.

Field vole (Fig. 6, ×6,100).

Fig. 1. Mucosal surface of the fundic stomach in the field vole. A number of oval gastric pits open on the mucosal surface. ×260.

Fig. 7. Mucosal surface of the glandular stomach in the golden hamster. There are some elongated gastric pits on the flat mucosa. Individual epithelial cells are recognized as short projections, as is the case in the field vole. ×2,600.

Fig. 8. Triangular fimbria between the esophageal sac and the fundic stomach. The mucosal surface of the fimbria is covered with a keratinized epithelium. ×90.

Fig. 9. Surface structure of the transitional region from the pyloric gland segment to the duodenum. The pyloric gland segment, provided with round gastric pits, transforms abruptly to the net-fused villi of the duodenum. ×260.
Fig. 11. Bacteria on the mucosal surface of the esophageal sac. The shapes of these bacteria are rod-like. ×850.

Fig. 12. Microorganisms on the mucosal surface of the pyloric gland segment. Numerous spheroidal microorganisms are observed eminently. These microorganisms, equipped with budding projections, are obviously different from those of the esophageal sac. ×2,600.

Figs. 13 and 14. Transmission electron micrographs.
Fig. 13. Epithelium of the pyloric stomach. Flat-tended and slender cells in the stratum granulosum do not have apparent nuclei, but have some degenerating mitochondria and kerato-hyalin granule (arrows). The oval cells in the stratum basale possess large and oval nuclei, scarce cytoplasm and well-developed intercellular bridges. ×3,000.

Fig. 14. A cell, which is loaded with granules in the basal part of the cell body, found in the pyloric gland segment. Such cells are frequently seen at the basal portion of the pyloric glands. ×9,000.

Figs. 15–18. Light micrographs.
Fig. 15. Esophageal sac of the field vole. The epithelium of the esophageal sac consists of keratinized stratified squamous cells. ×230.

Fig. 16. Fundic stomach of the field vole. The mucosa possesses three distinctive layers consisting of surface mucous cells, mucous neck cells, parietal cells and chief cells. ×70.

Fig. 17. Fimbria between the esophageal sac and the fundic stomach. The mucosa of the fimbria has a keratinized stratified squamous epithelium which continued from the esophageal sac. ×150.

Fig. 18. The mucosa of the pyloric gland segment. The epithelium is composed of one type of mucous cells. At the apical part, round microorganisms are recognized in large numbers. ×400.

Fig. 19. Surface mucous cells of the fundic stomach. The nuclei, scarce of chromatin, are located at the basale part of the cells. A number of tonofilbrils, amorphous structures and round small granules are recognized all over the cytoplasm. No apparent microvilli are observed on the free surface. ×9,000.

Fig. 20. Mucous neck cells of the fundic stomach. Mucous neck cells possess basally-located nuclei, numerous low electron-densed granules and some undeveloped microvilli. ×9,000.

Fig. 21. Parietal cell and chief cell of the fundic stomach. Parietal cell has the nucleus with scarce chromatin, many round mitochondria and numerous smooth endoplasmic reticula. Chief cell is provided with well-developed rough endoplasmic reticula and numerous zymogenic granules. The nucleus of chief cell abounds with chromatin, different from that of the parietal cell. ×6,000.

Fig. 22. Chief cell and its secretory granules. The granules of the chief cell are round, considerably variable in size and electron density. ×9,000.