Histological and Immunocytochemical Observation of the Hindstomach of the Collared Peccary, *Dicotyles tayacu* (Tayassuidae)

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ABSTRACT. Histology of the hindstomach mucosa of two collared peccaries, *Dicotyles tayacu*, was observed. The tissue samples were collected from 21 loci of the hindstomachs previously fixed and preserved in a buffered formalin. The pyloric mucosa is limited to a small area close to pylorus. Non-glandular mucosa is present in the region oral to the low folds which are continuous to the lips of the gastric groove. Cardiac gland exists microscopically between fundic and non-glandular epithelia and in the region close to the dorsal transverse fold. The fundic mucosa occupies most of the hindstomach mucosa and is twice as thick as the pyloric mucosa. Its thickness is 2.00 mm on an average. The fundic gland is a well-developed simple tubular type and the number of the gland cells per one-side of the tubule is as many as 503 on an average. In the very straight neck portion, a pair of the mucous neck cells or chief cells are closely associated with one parietal cell. Owing to the invagination in the isthmus, the parietal cells take the distinct distal position to the lumen. The general histology of the peccary fundic gland appears to be fundamentally similar to that of the pig, though the gland is more developed in the pig. Three kinds of endocrine cells (serotonin-storing EC cells, somatostatin-storing D cells and gastrin-storing G cells) are identified in the peccary hindstomach by the immunocytochemical method.

The collared peccary, *Dicotyles tayacu*, belongs to Tayassuidae in Suborder Suina. The peccary and the pig are both omnivorous, but their stomachs are quite different in gross structure. The collared peccary has a four-chambered compound stomach in contrast to the simple stomach of the pig. Recently Langer [14–16] gave a thorough description on the anatomy of the peccary stomach. His comparative anatomical works in Artiodactyla stomachs show that the compound stomach of the collared peccary presents an intermediate form between the simple stomach of the pig and the highly advanced one of the hippopotamas [13].

Langer [15] gave a detailed description

on the histology of the non-glandular epithelium of the peccary stomach, and presented a schema of the mucosa types in the four compartments of the stomach. He gave a short description of the histology of the glandular mucosa of the hindstomach of the peccary, but the data presented are not comparable with that known in the pig stomach.

The present study is planned, therefore, to provide further data on the histology of the hindstomach mucosa of the collared peccary, as a step in the comparative histology of the Artiodactyla stomachs.

MATERIALS AND METHODS

Hindstomachs of two adult female col-

lared peccaries, *Dicotyles tayacu*, were obtained from the collection of Dr. P. Langer at Institut für Anatomie und Zytobiologie der Justus-Liebig-Universität (Giessen, Federal Republic of Germany). The peccaries were originally collected by Dr. Langer in 1976 from an Experimental Station of the Arizona Cooperative Wildlife Research Unit, Tucson, Arizona, U.S.A. [14–16]. The peccaries were fed on domestic swine food pellets. The stomachs were fixed *in situ* by perfusion with the modified buffered formalin of Carson *et al.* [4]. Further data on the record of the animals and the anatomical procedures employed at the time of the initial collection are available elsewhere

[15, 16].

Tissue samples were taken from 21 loci of the hindstomach which was cut into halves along the greater curvature (Fig. 1). The sampling was made in the summer of 1981 from the organs preserved in cold fixatives. The samples were dehydrated and embedded in paraffin by the method of Möller [19]. Five-micron thick sections were stained by hematoxylin and eosin or by PAS-aurantia-hematoxylin [10]. The depth of the gastric pit and the length of the fundic gland tubules were measured by the aid of an ocular micrometer (Olympus OSM, Olympus Optic Co., Tokyo). The thickness of the non-glandular epithelium was measured in the same manner.

Cell count of the fundic gland: The number of the parietal cells per one-side of the fundic gland tubule was counted on the photomicrographs (final magnification of about $\times 1700$). The numbers of the mucous neck cells and/or chief cells were counted as follows: the numbers were directly counted on the photomicrographs with isthmus and base of the gland, and those in the neck were obtained by doubling the numbers of the parietal cells in this portion.

Immunocytochemistry of gastric endocrine cells: For gastric endocrine cells, deparaffinized sections were treated with Masson-Hamperl's argentaffin reaction [21] and Grimelius' argyrophil reaction [8]. Some section were treated immunocytochemically with unlabelled antibody methods [23] using antisera against gastrin, somatostatin, glucagon, glicentin, secretin, cholecystokinin, motilin and neurotensin. Controls were run as recommended by Sternberger [23].

RESULTS

1. Pyloric gland

It is a simple tubular gland with some

Fig. 1. The loci of tissue samples and mucosa types in the hindstomach.

1: right view of whole stomach, 2: internal aspect of caudodorsal face of hindstomach, 3: internal aspect of cranioventral face of hindstomach. C: cardiac mucosa, D: dorsal transverse fold, E: esophagus, F: fundic mucosa, GL: left lip of gastric groove, GP: gastric pouch, GR: right lip of gastric groove, HS: hindstomach, P: pyloric mucosa, PY: pylorus, S: stratified non-glandular mucosa, UB: upper blindsac, V: ventral transverse fold. 1–21: loci of tissue samples.

coilings and few branchings. From place to place several glands open into a common pit which penetrates halfway down into lamina propria. The majority of the glands open, however, into its own pit independently (Fig. 2). The ratio of the pit depth to the gland length in longitudinal sections is approximately 1:9. The pyloric gland cells contain PAS-positive mucin, but their nuclei are not completely flattened.

The pyloric mucosa is limited to the aboral area of the hindstomach close to the pylorus (Loci 1, 2, 3, 5 in Fig. 1). The average thickness of the mucosa is approximately 1.1 mm (Table 1). At Locus 2, the mucosa is pyloric in Peccary 1, but transitional between pyloric and fundic in Peccary 2 (Fig. 1).

2. Cardiac gland

It is a simple branched tubular gland with some coilings in the terminal portion (Fig. 3). Several glandular tubules open into a common pit which penetrates halfway down into lamina propria.

The cardiac glands are found microscopically between fundic and non glandular

epithelia. The average thickness of the cardiac mucosa (Loci 15, 16, 17 in Fig. 1) is approximately 0.9 mm (Table 1). In Peccary 2 it is a mixed cardiac mucosa in which a few parietal cells and some chief cells are present with a large number of cardiac gland cells (Fig. 1).

3. Non-glandular mucosa

It is covered by a stratified squamous epithelium with a good cornification (Fig. 4). Stratum granulosum is not evident due to the absence of large typical keratohyalin granules in the transitional zone between upper stratum spinosum and stratum corneum. In this zone some uppermost cells are swollen and clearer (Fig. 4). Retention of nuclei is commonly seen in lower stratum corneum. In the outer stratum corneum the adhesion of bacteria is also seen.

The non-glandular mucosa is limited to the area oral to the low folds which are continuous with the lips of the gastric groove (Fig. 1). The non-glandular epithelium (Loci 19, 20, 21) is thinner in Peccary 1 than in Peccary 2 (Table 2). The ratio of thickness of Stratum cor-

Table 1. Thickness of hindstomach mucosa (mm).

Pyloric gland mucosa		Cardiac gland mucosa		Fundic gland mucosa	
Locus	Length	Locus	Length	Locus	length
1	1.05	15	0.96	4	2.32
2	1.27	16	0.90	6	2.35
3	0.94	17	0.88	7	2.18
5	1.18			8	2.26
				9	1.92
Average		Average		10	1.81
±S.E.	1.11±0.17	±S.E.	0.91±0.04	11	2.37
				12	1.98
				14	2.01
				15	1.58
				18	1.19
				Average	
				±S.E.	2.00±0.37
				Average	
				±S.E.	2.13±0.21
				(4-14)	

neum to other strata of the epithelium is approximately between 1.31 and 1.50 (Table 2).

4. Fundic gland

A simple tubular fundic gland opens into its own pit. The pit depth is approximately 1/20 of the gland length. The gland is divided into three portions, isthmus, neck and base [9]. The isthmus has a wide lumen and distinguished from the pit by the presence of the parietal cells (Fig. 5). The lumen is narrower in the lower part of the isthmus, but is still wider than that of the neck. The mucous neck cells are present in the upper part of the neck, while typical chief cells are present in the lower part of the portion (Figs. 6, 7). The mucous neck cells are also present in the lower neck and in the base (Fig. 7). The base is distinguished

from the neck by the extensive coiling of the gland tubules and by the disappearance of parietal cells (Fig. 7). Few branchings are observed in the base. The ratio of the length in these three portions is 1.0:4.0:1.6 for the isthmus, neck and base, respectively. The neck occupies 65% of the whole gland length (Table 3).

The fundic mucosa is present in most of the area aboral to the dorsal and ventral transverse folds (Loci 4, 6–15, 17, 18 in Fig. 1). The average mucosa thickness is approximately 2.0 mm (Table 1). The mucosa is thin at Loci 15 and 18.

5. The cellular configuration of the fundic gland

In the isthmus, the glandular epithelium assumes a simple layer in which the parietal cells directly face the gland lu-

Table 2. Thickness of non-glandular epithelium and ratio of stratum corneum to other strata of the epithelium. (Average $\pm$ S.E.)

Peccary No.	Locus	Average thickness (mm)			Average ratio (A/B)
		Stratum corneum (A)	Other strata (B)	Total	
1	19	0.31	0.28	0.59	1.44
	20	0.32	0.31	0.63	1.02
	21	0.39	0.29	0.68	1.47
	Average	0.34 $\pm$ 0.12	0.29 $\pm$ 0.02	0.63 $\pm$ 0.04	1.31 $\pm$ 0.25
2	20	0.98	0.53	1.51	1.96
	21	0.48	0.56	1.04	1.03
	Average	0.73 $\pm$ 0.35	0.55 $\pm$ 0.02	1.28 $\pm$ 0.33	1.50 $\pm$ 0.66

Table 3. Relative length of the three portions of the fundic gland and cell numbers per one-side of the gland tubules

Locus	Relative length (%)			Gland cell number: parietal cell (other gland cells)			
	Isthmus	Neck	Base	Isthmus	Neck	Base	Total
4	15.3	62.5	22.2	10(20)	125(250)	20(80)	155(355)
6	16.0	63.8	20.2	15(40)	132(264)	22(80)	169(384)
7	7.8	64.9	27.3	6(20)	110(220)	12(55)	128(295)
9	14.9	70.2	14.9	16(30)	138(276)	16(50)	170(356)
Average $\pm$ S.E.	13.5 $\pm$ 3.8	65.4 $\pm$ 3.4	21.2 $\pm$ 5.1	11.8 $\pm$ 4.7 (27.5 $\pm$ 9.6)	126.3 $\pm$ 12.1 (252.5 $\pm$ 24.2)	17.5 $\pm$ 4.4 (66.3 $\pm$ 16.0)	155.5 $\pm$ 9.6 (347.5 $\pm$ 37.5)

men. In the lower isthmus, however, an epithelial invagination takes place (Fig. 8). The invagination is also seen in the neck where the epithelium assumes a "double layered" appearance. Namely the mucous neck cells and chief cells are at the "first" layer, while parietal cells form apparent "second" layer (Figs. 6, 9, 10). As observed commonly, one parietal cell is closely associated with a pair of other gland cells (Figs. 9, 10). In a plane of section through the "first" layer such pairs are clearly seen (Fig. 11).

6. The cell number of the fundic gland

The average number of the parietal cells per one-side of the gland tubule in four loci (Loci 4, 6, 7, 9) is 11.8, 126.3 and 17.5 at the isthmus, neck, and base, respectively (Table 3). The average number of the mucous neck cells and/or chief cells at these three portions is 27.5, 252.5 and 66.3, respectively (Table 3). The total number of the gland cells per one-side of the gland tubule is approximately 503 on an average (Table 3).

7. Lamina propria

Large collecting venules are present in lamina propria (Fig. 7). In the gastric pit region, subepithelial networks of capillaries are also seen (Fig. 5). The gland tubules are separated by connective tissue elements of a certain amount. They do not form conspicuous septum-like structure (Fig. 6).

8. Gastric endocrine cells

Both argentaffin and argyrophil cells are detected in the hindstomach of the

peccary. They are scattered throughout the pyloric glands from neck to bottom. In the cardiac and fundic glands, however, they are found predominantly in the basal half of the mucosa (Figs. 12, 13). The argyrophil cells are numerous in the pyloric and fundic glands and few in the cardiac glands (Table 4). Relative frequency of the argentaffin cells is lower than that of the argyrophil cells, especially in the fundic and pyloric glands (Table 4).

Immunocytochemically, somatostatin-immunoreactive cells (somatostatin cells or D cells) and gastrin-immunoreactive cells (gastrin cells or G cells) are detected in the peccary hindstomach, but other kinds of immunoreactive cells are not identified in this study. The somatostatin cells are numerous in the pyloric glands (Fig. 14), less so in the fundic glands (Fig. 14) and few in the cardiac glands (Table 4). Numerous gastrin cells are found only in the pyloric glands (Fig. 15 and Table 4). These immunoreactive cells show some differences in their topographical distribution in the three regions of the hindstomach. The somatostatin cells are scattered throughout the pyloric glands from neck to bottom. In the cardiac and fundic glands, however, they are mainly found in the basal half of the mucosae. The gastrin cells are found in the middle third of the mucosa.

The endocrine cells in the cardiac and pyloric mucosae appear to possess an apical cytoplasmic process in contact with

Table 4. Relative frequency of the endocrine cells

Cell type	Cardiac glands	Fundic glands	Pyloric glands
Argentaffin cells	+	+	+
Argyrophil cells	++	+++	+++
Somatostatin cells	+	++	+++
Gastrin cells	—	—	+++

—: absent, +: few, ++: moderate, +++: numerous

the glandular lumen (Fig. 16), while in the fundic glands they have no contact with the lumen (Fig. 17). The formers are located among the exocrine cells and are mainly pyramidal or barrel-like in shape. The latters are situated between the exocrine cells and the basal lamina, and show an ellipsoid or elongated shape.

DISCUSSION

The distribution of the mucosa types in the hindstomach of the collared peccary confirms the schema of the distribution given by Langer [15]. The fundic mucosa occupies most of the hindstomach area and is twice as thick as the pyloric mucosa. The short pit and distinct isthmus suggest that the fundic gland is well-developed in the collared peccary.

Archer[2] reported that the thickness of the fundic mucosa is about 1500 μm to 2000 μm in the pig aged from 5 to 44 months. The ratio of the thickness of the fundic to pyloric mucosa is between 1.5 to 2.0 [2]. Kondo [12] reported that the pig fundic mucosa is twice as thick as that of the pyloric mucosa, the average thickness of the former being 1838 μm . The ratio of the gastric pit depth to the gland length of the fundic gland is 1:5.3 [12]. The "double layered" appearance of the fundic gland epithelium has been reported in the pig [2, 22].

The comparison of the above data on the histology of the fundic glands shows that the gland in the collared peccary has certain similar characteristics to those in the pig. The presence of the mucous neck cells in the lower portion of the fundic gland tubules is another common characteristic in both species, an unusual property in other mammalian fundic gland [9, 22]. The thicker mucosa and relatively long gland tubule in the pec-

cary suggest, however, that the gland is more developed in the peccary than in the pig.

The pyloric gland of the collared peccary is less branched than in the pig. In the pig, the pyloric mucosa is 0.9 mm to 1.5 mm thick [2, 22]. These data show that the pyloric gland is more developed in the pig than in the collared peccary in contrast to the trend observed in the fundic gland.

In spite of numerous reports on the histology of the fundic gland, the extensive invagination of the fundic epithelium and the quantitative association of the parietal cells to other gland cells have not been reported [9]. The similarity of the peccary fundic gland to that of the pig suggests that such special features may be common in the fundic glands of other members of Suborder Suina.

The cell number of the fundic gland has been reported by several workers in various species [5, 7, 9, 11]. In the cow the cell number per gland is 130, including 39 parietal cells [11]. In the mouse the number per gland is 82, including 16 parietal cells [5]. In the meadow vole (*Microtus pensylvanicus*), the total cell number of the fundic gland plus surface epithelial cell is about 60–70 [7]. The number of the gland cells in the collared peccary is as many as 503 per one-side of the gland. The above data show that the peccary fundic gland has an extraordinary large number of the gland cells. The very thick mucosa and the "double layered" configuration of the gland cells may be responsible for this phenomenon.

Due to the coiling of the gland tubules at the base, the cell counts presented here are only approximates. The workers who gave the fundic gland cell counts used different methods which were usually not clearly stated in their papers.

Thus the use of the gland cell counts in comparative histology of the fundic glands remains to be examined in further studies.

In the peccary hindstomach, argentaffin and argyrophil cells were detected histologically, and somatostatin and gastrin cells were identified immunocytochemically. The argentaffin cells are also called as the enterochromaffin cells (EC cells) which synthesize and store serotonin [6]. Therefore, there are three kinds of endocrine cells in the peccary hindstomach; serotonin-storing EC cells, somatostatin-storing D cells and gastrin-storing G cells. The distribution and frequency of the endocrine cells in the peccary hindstomach appear to be basically similar to those in the pig [1, 3, 18, 20]. Larson *et al.* [17] reported that glucagon-immunoreactive cells were more numerous in the cardiac glands than in the fundic glands of the pig. In the peccary, however, neither glucagon-immunoreactive cells nor glicentin-immunoreactive cells are detected. This difference between pig and peccary may be due to the difference of fixatives and/or fixation methods used. It is well known that somatostatin cells show the widest distribution in the gastrointestinal tract. Somatostatin cells are more numerous in the fundic glands than in the pyloric glands, whereas the reverse was true for the cat, dog and man [1]. In the peccary hindstomach, somatostatin cells are more numerous in the pyloric glands than in the fundic glands. The significance of these differences between the peccary and the pig glands is not clear at present.

Electronmicroscopically, five types of endocrine cells (EC, ECL, D, D₁ and G cells) were identified in the pig stomach [3]. In the peccary hindstomach, However, only three types of endocrine cells

(EC, D and G cells) are detected histologically and immunocytochemically. Since ECL and D₁ cells can be identified only by ultrastructural study the presence of these two types remains to be investigated.

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EXPLANATION OF FIGURES

Figs. 2–11. PAS-aurantia-hematoxylin stain.

Fig. 2. Pyloric mucosa. Locus 3. Simple tubular pyloric glands with few branchings and coilings. Some tubules open into a common gastric pit (star). $\times 100$.

Fig. 3. Cardiac mucosa. Locus 17. Simple tubular cardiac glands, some of them open into a common gastric pit (star). $\times 100$.

Fig. 4. Non-glandular mucosa. Locus 19. Thick stratum corneum retains nuclei in the lower layer. Stratum granulosum is not evident. Some cells (arrow) in the transitional zone from stratum spinosum to stratum corneum are swollen and clearer. $\times 200$.

Fig. 5. Gastric pit (asterisk) and isthmus (star). At isthmus the gland lumen is wide and parietal cells (arrow) are present. The epithelium assumes a simple layered configuration. $\times 200$.

Fig. 6. Upper neck. The glandular epithelium assumes a "double layered" configuration. Mucous neck cells and parietal cells take "proximal" and "distal" position to the lumen, respectively. $\times 400$.

Fig. 7. Base. A few mucous neck cells (arrow) are present among chief cells and few parietal cells. Coiling of gland tubules is noted. Large collecting venule (asterisk) is seen. $\times 200$.

Fig. 8. Lower isthmus. Epithelial invagination (arrow) results in the distal replacement of parietal cells in the distal layer. $\times 400$.

Figs. 9–11. Neck. One parietal cell in the distal layer is associated with a pair of mucous neck cells (short arrow). An epithelial invagination

(long arrow) is noted. $\times 750$.

Fig. 11. A section through a plane at the proximal layer of the epithelium. Mucous neck cells are present in pairs.

Fig. 12. Fundic mucosa. Locus 11. A small number of argentaffin cells (EC cells) are found in the basal half of the mucosa. Masson-Hamperl's argentaffin reaction. $\times 180$.

Fig. 13. Serial section of the Fig. 12. Numerous argyrophil cells are found in the basal half of the fundic mucosa. Their cytoplasmic processes never reach the glandular lumen. Grimelius argyrophil reaction. $\times 180$.

Fig. 14. Pyloric mucosa. Locus 3. Numerous somatostatin cells (D cells) are found in the basal half of the mucosa. Some cells reach their cytoplasmic processes to the glandular lumen. Unlabelled antibody method. $\times 180$.

Fig. 15. Serial section of the Fig. 14. Gastrin cells are found in the middle third of the pyloric mucosa. The cytoplasmic processes of some cells reach the glandular lumen. Unlabelled antibody method. $\times 180$.

Fig. 16. Pyloric mucosa. Locus 3. Two gastrin cells (G cells) in the pyloric glands. One reaches the glandular lumen with its apical cytoplasmic process (arrow). Unlabelled antibody method. $\times 600$.

Fig. 17. Fundic mucosa. Locus 11. Two somatostatin cells (D cells) in the fundic glands. Their cytoplasmic process extend along the basal lamina. They have no contact with the glandular lumen. Unlabelled antibody method. $\times 600$.

要 約

クビワペッカリ, *Dicotyles tayacu* (Tayassuidae) の後胃の組織学的ならびに免疫組織化学的研究: 玉手英夫・山田純三¹⁾ (東北大学農学部家畜形態学教室, ¹⁾帯広畜産大学家畜解剖学教室)——2頭のクビワペッカリ, *Dicotyles tayacu* 後胃を緩衝ホルマリンで灌流固定して観察した。幽門腺部は幽門近い小部分に限られ、非腺部は胃溝の唇部に続く低いひだの口方に存在した。噴門腺は胃底と非腺部上皮の中間に顕微鏡的に存在するほか、背側横ひだに近い部分にも存在した。胃底腺部粘膜は後胃粘膜の大半を占め、幽門腺部粘膜より約2倍厚く、平均 2.00 mm であった。胃底腺は発達した単管状腺で腺管の1側の腺細胞数は平均 503 個に達し、直走する腺頸部では2個の副細胞または主細胞が1個の壁細胞と密接に対応していた。腺峡部上皮の陥入により壁細胞は腺腔に対して明らかな遠位性を示した。ペッカリー胃底腺はブタ胃底腺と基本的には同様の構造であるが、より発達していた。幽門腺はブタにくらべて発達がよくなかった。免疫組織化学的方法により3種類の内分泌細胞(セロトニン貯蔵細胞, ソマトスタチン貯蔵細胞, ガストリン貯蔵細胞)がペッカリー後胃組織で証明された。