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An histochemical study of endocrine cells in the alimentary tract of the red-eared slider, *Trachemys scripta elegans*

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Abstract : The regional distributions and relative frequencies of endocrine cells were studied histochemically (Grimelius and Masson-Hamperl (M-H) silver methods) in the alimentary tract of the red-eared slider, *Trachemys scripta elegans* . Samples were taken from the esophagus, fundus, pylorus, duodenum, jejunum, ileum and large intestine. Argyrophil (Grimelius-positive) cells and argentaffin (M-H-positive) cells were found in the whole alimentary tract in this study. Spherical to spindle and/or oval to round-shaped argyrophil or argentaffin cells were located in the gastric glands of the stomach regions, in the basal portion of the epithelium of intestinal tract or the esophagus with variable frequencies. Argentaffin cells were more numerously detected in the whole alimentary tract compared with those of argyrophil cells in this study.

Argyrophil cells were observed in the whole alimentary tract including the esophagus and the most predominant region was the rectum with moderate frequency. The relative frequency of these cells was rare in the esophagus, fundus, duodenum, jejunum and ileum, respectively and a few frequency in the pylorus.

Argentaffin cells were also observed in the whole alimen-tary tract including the esophagus and the most numerously demonstrated region was the rectum with numerous frequency. They were observed with a few frequencies in the remaining regions of the alimentary tract except for the rectum, respectively.

However, to know the exact type of the argyrophil cells and argentaffin cells that were observed in this study, more developmental methods such as immunohistochemistry were needed.

Key words : endocrine cells, alimentary tract, red-eared slider, histochemistry, silver techniques.

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Introduction

The red-eared slider, *Trachemys scripta elegans*, belonging to the Emydidae in order Testudines habitating south part of America and it is also called Florida turtle because of their habitation regions. Recently, this slider has been highlighted as a pet animal in the worldwide including Korea. On the other hand, it is a problem that the neglected sliders once reared as a pet caused the destruction of ecosystem in non-natural habituated Korea. Gastrointestinal endocrine cells dispersed in the epithelia and gastric glands of the alimentary tract synthesized various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract[1]. Until now, the investigation of gastrointestinal endocrine cells is considered to be an important part of a phylogenic study[2]. In addition, the regional distributions and relative frequencies of these endocrine cells were varied with animal species and feeding habits[3]. Although many studies have elucidated the regional distribution and relative frequency of different endocrine cells in the alimentary tract of the various vertebrates, studies on the reptilia have received little attention. Most recently intensive studies have been done on the reptilian species because their phylogenetical tree is situated in middle of the evolution of vertebrates[4].

Grimelius and Masson-Hamperl (M-H) silver methods were widely used as a method for identification of gastrointestinal endocrine cells[5,6] but untill now, the chemical characteristics of the Grimelius-positive argyrophil cells remained as unsolved problem[7,8]. Kotze *et al*[9]. reported that the intestinal tract of the Nile crocodile (*Crocodylus niloticus*) is shown to be line with the situation in crocodilia and also exhibits a resemblance to that of carnivorous mammals. Pastor *et al*[10]. suggested that the tracheal epithelium of *Testudo graeca* and *Pseudemys scripta elegans* consisted of three cell types- mucous, ciliated and basal- and the Grimelius silver argyrophil technique was positive in a population of tracheal cells. In addition, the appearance of argentaffin and argyrophil cells in the intestines of *Xenodon merremii* was demonstrated[11]. But little data is available on the regional distribution and relative frequency of endocrine cells along the entire length of the gastrointestinal tract (GIT) including the esophagus of the Emydidae.

The purpose of the present study was to clarify the regional distribution and relative frequency of the endocrine cells in the alimentary tract of the Emydidae, *Trachemys scripta elegans* by Grimelius and M-H silver methods.

Materials and Methods

Five adult (16~20cm in diameter) red-ear sliders of the Emydidae, *Trachemys scripta elegans*, were captured in the Kyungsan and were used in this study without sexual distinction. The animals were anesthetized with ethyl ether. As there was no clear demarcation between the regions of the large intestinal portions differently from mammals, the alimentary tract of the red-eared slider was divided into 7 portions (esophagus, fundus, pylorus, duodenum, jejunum, ileum and rectum) modified the division of the desert tortoise (*Xerobates agassizii*)[12]. After phlebotomized, samples from the esophagus, fundus, pylorus, duodenum, jejunum, ileum and rectum were fixed in Bouin's solution. After paraffin embedding, 3~4μm serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal alimentary architecture.

Each representative sections were deparaffinized, rehydrated and silver stained with the Grimelius methods[6] for argyrophil cell identification, and M-H methods[5] for argentaffin cell identification.

The regional distribution and relative frequency of the Grimelius-positive argyrophil cells and M-H-positive argentaffin cells were observed under light microscropy.

Result

Spherical to spindle and/or oval to round-shaped argyrophil or argentaffin cells were located in the gastric glands of stomach regions, in the basal portion of the epithelium of intestinal tract or esophagus with variable frequencies. Argentaffin cells were more numerously detected in the whole ali-

mentary tract compared with those of argyrophil cells in this study. The regional distribution and relative frequency of argyrophil and argentaffin cells were showed in Table 1.

Argyrophil cells were demonstrated in the whole alimentary tract with various relative frequencies. Spindle shaped argyrophil cells were found in the interepithelial cells in the epithelium of the esophagus with rare frequency (Fig 1a). Spherical to spindle shaped cells were detected in the basal portion of the surface epithelium of the fundus but oval to round shaped cells were observed in the gastric glands. They were demonstrated in this region with rare frequency (Fig 1b). Similary to the frequency of the fundus, spherical to spindle shaped cells were detected in the basal portion of the surface epithelium of the pylorus but oval to round shaped cells were observed in the gastric glands. They were demonstrated in this region with a few frequency (Fig 1c). Spherical to spindle shaped cells were detected in the basal portion of the simple columnar epithelium of the duodenum with rare frequency (Fig 1d). The shape, relative frequency and regional distribution in the jejunum were similar to that of the duodenum (Fig 1e). Spherical to spindle shaped cells having long cytoplasmic process that was extended toward luminal cavity were observed in the basal portion of the epithelium of the ileum with rare frequency (Fig 1f). In the rectum, which was the most predominant regions in this study with moderate frequency, various shaped cells were detected in the interepithelial cells in the surface epithelium (Fig 1g).

Argentaffin cells were demonstrated in the whole alimentary tract with various relative frequencies. Spindle shaped argentaffin cells were found in the interepithelial cells in the epithelium of the esophagus with a few frequency (Fig 2a). Spherical to spindle shaped cells were detected in the basal portion of the surface epithelium of the fundus but oval to round shaped cells were observed in the gastric glands. They were demonstrated in this region with a few frequency (Fig 2b). Similarly to the frequency of the fundus, spherical to spindle shaped cells were detected in the basal portion of the surface epithelium of the pylorus but oval to round shaped cells were observed in the gastric glands. They were demonstrated in this region with a few frequency (Fig 2c). Spherical to spindle shaped cells, among those of cells some cells having long cytoplasmic process that was extended toward luminal cavity were existed, detected in the basal portion of the simple columnar epithelium of the duodenum with a few frequency (Fig 2d). The shape, relative frequency and regional distribution in the jejunum and ileum were similar to those of the duodenum (Fig 2e, f). In the rectum, which was the most predominant regions in this study with numerous frequency, various shaped cells were detected in the interepithelial cells in the surface epithelium (Fig 2g).

Discussion

The endocrine cells in the alimentary tracts appeared remarkably different in the regional distribution, relative frequency and cell types with animal species and each regional part of the digestive tract[13,14]. Until now, the regional distribution and relative frequency of the endocrine cells that were located in the GIT were extensively studied by various methods including histochemistry, immunohistochemistry and ultrastructure but the most classical methods for identifying the endocrine cells in the alimentary tract was silver impregnate methods[5-8]. Most of endocrine cells located in the GIT

Table 1. The regional distributions and relative frequencies of the endocrine cells in the alimentary tract of the red-eared slider. *Trachemys scripta elegans*

	Esophagus	Fundus	Pylorus	Duodenum	Jejunum	Ileum	Rectum
Argyrophil cell[1)]	±*	±	+	±	±	±	++
Argentaffin cell[2)]	+	+	+	+	+	+	+++

[1)] Grimelius-positive cells, [2)] Masson-Hamperl-positive cells,
*Relative frequencies; +++ : numerous, ++ : moderate, + : a few, ± : rare.

were subdivided to types by silver impregnate methods[5-8]. Namely, they were Grimelius-positive argyrophil cells and M-H-positive argentaffin cells and especially, these two methods have been used widely in the identification of the endocrine cells in the GIT and panceas but these cells were showed quite different patterns in the regional distribution and relative frequency[15,16].

About silver staining of the endocrine cells, Kim[17] asserted that argyrophil cells should be more numerously demonstrated than argentaffin cells in rat and human GIT and they also insisted that the appearance of argyrophil cells should be more rapidly observed than those of argentaffin cells at the stages of ontogeny. In addition, the relative frequencies of these cells were changed to similarly step-by-step during ontogeny, and it was observed that a sudden increase of these two types of endocrine cells was detected after birth. Chung and Kwun[18] reported that argyrophil cells were numerous and argentaffin cells were found in the fundus while rare or a few argentaffin cells were found in this region, but they persisted that argyrophil cells should be not observed in the intestine. Also Chung[19] reported that these two types endocrine cells were observed in the basal portion of the fundus and in the small intestine, these cells were detected more numerously than the frequency of the large intestine.

In the present study, argentaffin cells were more numerously detected in the whole alimentary tract compared with the frequencies of argyrophil cells in this study and these cells were more numerously detected in the large intestine compared with the frequencies of the small intestine. The regional distribution and relative frequency of these cells in the fundus were quite different from those of the Chung and Kwun[18], and the appearance of argyrophil and argentaffin cells in the intestinal tract of the slider was different from the results of Chung and Kwun[18] who reported that argyrophil cells were not detected in the intestine. Deduce from El-Sahly and Grimelius[20] who suggested that the methods used at sampling could influence the appearance of endocrine cells in the GIT, it is considered that these differences were not originated from absence of these argyrophil cells in the intestine but fixation problem. Quite differently from the previous report[17,21], argentaffin cells were more numerously detected in the whole alimentary tract compared with those of argyrophil cells in this study. These differences were considered, as species-specific characteristic problem of Emydidae but it is difficult to conclude because of the absence of other available data about the argyrophil and/or argentaffin cells in the alimentary tract of the other Emydidae. However, similarly to Solcia *et al*[3]. who reported that argyrophil and argentaffin cells were used as a identification index of non-amine endocrine cells in the GIT, these cells were observed in the whole GIT including the esophagus. The results that argyrophil and argentaffin cells were more numerously detected in the large intestine comparing with to the frequencies of the small intestine in this study were well correspond to those of the previous reports[19,21].

Anyway, the shape of the endocrine cells in the GIT was reported that oval to round close type cells were demonstrated in the gastric gland of the fundus and pylorus, and spherical to spindle shaped open type cells were mainly located in the epithelium of the intestinal tract[3,22]. In the present study, spherical to spindle shaped cells were observed in the interepithelia of the surface epithelium of the intestinal tract and oval to round cells were restricted to the gastric glands of the fundus and pylorus. These results were similar to those of other mammalian species[3,22-25].

In conclusion, spherical to spindle and/or oval to round-shaped argyrophil or argentaffin cells were located in the gastric glands of stomach regions, in the basal portion of the epithelium of intestinal tract or esophagus with variable frequencies. Argentaffin cells were more numerously detected in the whole alimentary tract comparing with those of argyrophil cells in this study. However, to know the exact type of the argyrophil cells and argentaffin cells that were observed in this study, more developmental methods such as immunohistochemistry were needed.

Legends for figures

Fig 1. Argyrophil cells in the alimentary tract of the red-eared slider.

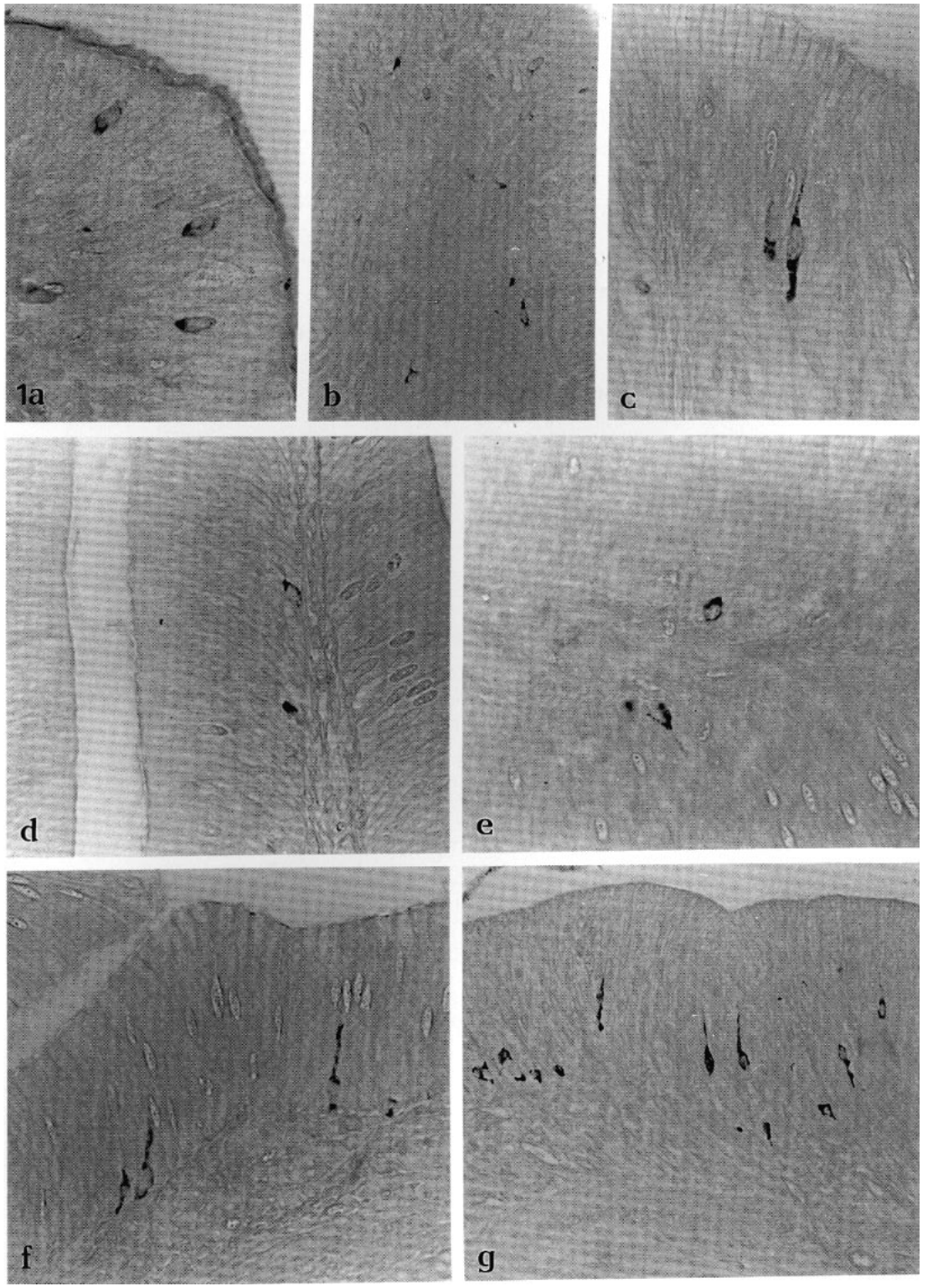
a. Esophagus	b. Fundus	c. Pylorus	d. Duodenum
e. Jejunum	f. Ileum	g. Large intestine	

a, c-f : ×480 ; b, g : ×240, Grimelius methods

Fig 2. Argentaffin cells in the alimentary tract of the red-eared slider.

a. Esophagus	b. Fundus	c. Pylorus	d. Duodenum
e. Jejunum	f. Ileum	g. Large intestine	

a-f : ×480 ; g : ×240, Masson-Hamperl methods


1a
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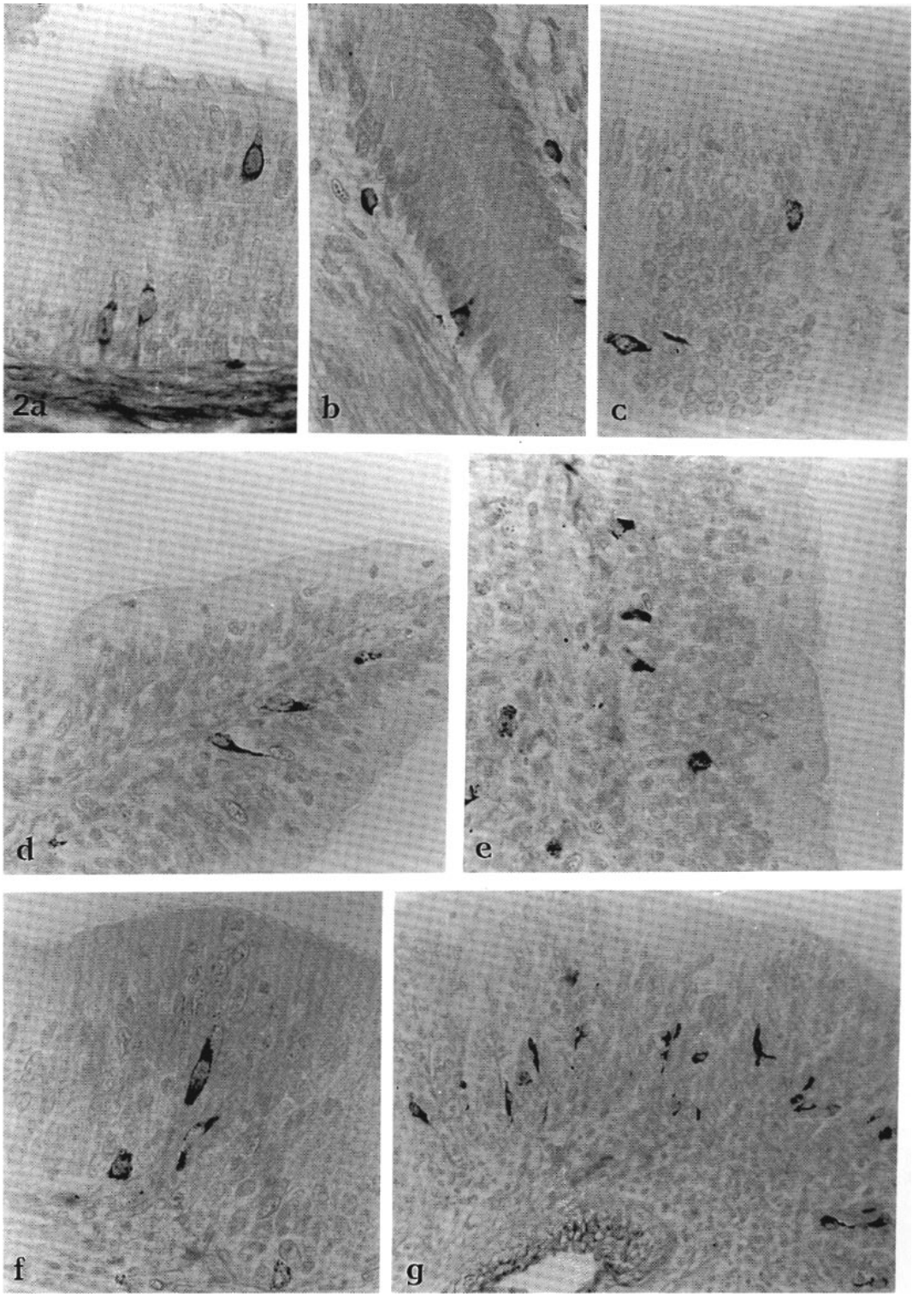
2a
b
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g

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붉은귀거북이 소화관 내분비세포에 관한 조직화학적 연구

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국문초록 : 붉은귀거북이(red-eared slider, *Trachemys scripta elegans*)의 소화관에 존재하는 위장관내분비세포의 부위별 분포 및 출현빈도를 Grimelius 및 Masson-Hamperi(M-H) 도은염색을 이용하여 조직화학적으로 관찰하였다. 소화관은 식도, 기저부(fundus), 유문부, 십이지장, 공장, 회장 및 대장의 7부위로 구분하였다. 소화관 전 부위에 걸쳐 은호성세포(argy-rophil, Grimelius-positive cell) 및 은친화성세포(argentaffin cell, M-H-positive cell) 들이 관찰되었다. 타원형에서 방추형 또는 난원형에서 원형의 은호성세포 및 은친화성세포들이 기저부와 유문부의 위샘(gastric gland), 장 및 식도상피의 기저부에서 관찰되었으며, 소화관 각 부위별로 다양한 출현빈도를 나타내었다.

은호성세포들은 식도를 포함한 소화관 전 부위에서 관찰되었으며, 중등도의 출현빈도를 나타낸 직장에서 가장 높은 빈도를 나타내었고 식도, 기저부, 십이지장, 공장 및 회장에서 극소수, 유문부에서는 소수 관찰되었다.

은친화성세포들 역시 소화관 전 부위에서 관찰되었으며, 은호성세포와 유사하게 다수의 출현빈도를 나타낸 직장에서 가장 높은 빈도를 나타내었고, 직장 이외의 부위에서는 소수의 세포들이 관찰되었다.

Key words : endocrine cells, alimentary tract, red-eared slider, histochemistry, silver techniques.