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# Distribution of endocrine cells in the digestive tract of *Alligator sinensis* during the active and hibernating period

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ABSTRACT

The digestive tract is the largest endocrine organ in the body; the distribution pattern of endocrine cells varies with different pathological and physiological states. The aim of the present study was to investigate the distributed density of 5-hydroxytryptamine (5-HT), gastrin (GAS), somatostatin (SS) and vasoactive intestinal peptide (VIP) immunoreactive (IR) cells in the digestive tract of *Alligator sinensis* during the active and hibernating period by immunohistochemical (IHC) method. The results indicated that 5-HT-IR cells were distributed throughout the entire digestive tract, which were most predominant in duodenum and jejunum. The density increased significantly in stomach and duodenum during hibernation. GAS-IR cells were limited in small stomach and small intestine. The density decreased significantly in small stomach during hibernation, while increased in duodenum. What's more, most of the endocrine cells in duodenum were generally spindle shaped with long cytoplasmic processes ending in the lumen during hibernation. SS-IR cells were limited in stomach and small stomach. The density increased in stomach while decreased in small stomach during hibernation, meanwhile, fewer IR cells occurred in small intestine. VIP-IR cells occurred in stomach and small stomach. The density decreased in small stomach, while increased in stomach during hibernation. These results indicated that the endocrine cells in different parts of digestive tract varied differently during hibernation, their changes were adaptive response to the hibernation.



## 1. Introduction

The digestive tract is the largest endocrine organ in the body (Buddington and Krogdahl, 2004). The endocrine cells are dispersed along the epithelium, gastric and intestinal glands of the digestive tract and secrete various types of gastrointestinal hormones to regulate many physiological processes, such as the control of motility, the secretion of fluid, electrolytes and digestive enzymes, cell proliferation and survival. It has been suggested that the distribution pattern of endocrine cells in the digestive tract might result from natural selection during evolution to adapt to specific feeding habits, which could be altered by various factors such as food composition, pathological variations, starvation, or environmental conditions (Lee and Ku, 2004; Yang and Wang, 1997; Pan et al., 2009).

Hibernation is a survival strategy for animals to cope with the low temperature and food restriction of winter (Andrews, 2007). Hibernators exhibit profound physiological changes including low body temperature, respiratory depression, bradycardia, analgesia, and attenuated general metabolism. During hibernation, the animals cease feeding, the physiological activities of the digestive tract decrease significantly, while the overall architecture of the gut epithelium is well preserved with enterocyte microvillus height unchanged and microvillus density slightly increased, the epithelial function is also well maintained (Carey et al., 2001). Xie et al. (2012) detected argentaffine cells in different parts of the digestive tract of Chinese Fire-bellied Newt (*Cynops orientalis*, David, 1873), totally the density of which was higher in hibernation than that in non-hibernation. However, up to date, little is known about the changes of the other types of endocrine cells in the digestive tract.

Chinese alligator (*Alligator sinensis*) belongs to alligatoridae family, crocodylia of reptilia, which is an endemic rare species in China and is one of the most endangered freshwater crocodilian species with an important ecological and economic value. Currently, there

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are ~100 Chinese alligators in the wild and ~10,000 captive individuals in Anhui and Zhejiang Provinces (Wan et al., 2013). Wu et al. (1999) have been identified four types of endocrine cells in the digestive tract of *A. sinensis*, they were 5-hydroxytryptamine (5-HT), gastrin (GAS), somatostatin (SS) and vasoactive intestinal peptide (VIP) immunoreactive (IR) cells, however, the exact time of the alligators used in the study was not mentioned. In this study, we investigated the distributed density of these four types of endocrine cells during the active and hibernating period by immunohistochemical (IHC) method.

The aim of the present study was to explore the density and morphology changes of endocrine cells during hibernation, the results could improve our understanding of digestive functions of Chinese alligators in different physiological states, and provide basic data for its artificial breeding and ecological protection, and enrich the knowledge of comparative endocrinology.

## 2. Materials and methods

### 2.1. Specimens and tissue sections

Six adult Chinese alligators (1.3–1.4 m in length) were provided by Xuancheng Alligator Culturing Centre. Three of them collected from May to September 2011 were active; the other three collected from December 2011 to February 2012 were at hibernating state. All procedures were approved by the forestry authorities of China.

The alligators were anaesthetized with an intraperitoneal injection of pentobarbital, and the digestive tract was rapidly removed. Samples taken from nine parts of the digestive tract (esophagus, cardia, fundus, pylorus, small stomach, duodenum, jejunum, ileum and rectum) were cleaned with 0.70% physiological saline, and then fixed in Bouin's fluid for 48 h, and dehydrated through graded ethanol, cleared in xylene and embedded in paraffin. Finally, 5 μm thick sections were obtained mounted on gelatin-coated slides and processed for IHC staining.

### 2.2. Antisera and reagents

The details of four types of antisera are listed in Table 1. Streptavidin–biotin–peroxidase complex (SABC) IHC kit was purchased from Wuhan Boster Corp (Wuhan, China).

### 2.3. Immunohistochemical staining

Each representative section was deparaffinized, rehydrated and immunostained with conventional methods. Endogenous peroxidase activity was blocked by incubating the sections with 3% $H_2O_2$ for 20 min. Washed in distilled water and then treated with phosphate-buffered saline (PBS, 10 mM, pH 7.4) for 5 min. Blocking of the non-specific reaction was performed with normal goat serum prior to overnight incubation at 4 °C with the primary antiserum (Table 1). After rinses in PBS (3× 5 min), the sections were incubated at room temperature with biotinylated goat anti-rabbit IgG secondary antibody for 2 h. After washing with PBS and subsequent incubation for 2 h, the SABC was applied. After washes, the peroxidase reaction was carried out in 3,3′-diaminobenzidine (DAB) solution containing 0.01% of $H_2O_2$ in Tris–HCl buffer (50 mM, pH 7.6) and subsequently washed with distilled water. The sections were lightly counterstained with Mayer's hematoxylin, dehydrated in serial alcohol, cleared in xylene and cover-slipped. Negative control was carried out by incubating sections with PBS instead of the primary antiserum. The positive control was performed in pathological section of human gastrointestinal tract. The IR cells were observed under light microscope.

**Table 1**
Details of the gut hormone antisera.

| Hormone antisera | Code No. | Dilution | Source |
|---|---|---|---|
| Rabbit anti-Serotonin(5-HT) | ZA-0231 | 1:100 | Zymed Corp |
| Rabbit anti-Gastrin (GAS) | ZA-0115 | 1:75 | Zymed Corp |
| Rabbit anti-Somatostain (SS) | ZA-0232 | 1:100 | Zymed Corp |
| Vasoactive intestinal peptide (VIP) | BA0134 | 1:100 | Boster Corp |

### 2.4. Observation, photomicrography and cell counting

For each tissue, five sections were randomly chosen. All sections were examined and images captured under Olympus BX61 microscope. The average number of positive cells from 20 randomly selected fields was determined and used in data evaluation, the enlargement of the field was 400×. The data were expressed as means ± standard errors (means ± SE) and the variance analysis was performed by SPSS 17.0 software. Statistical analysis was done with One-Way ANOVA in the case of comparison of multiple groups. Differences with $P < 0.05$ were considered significant.

## 3. Results and analysis

The IR cells present dark brown or yellow brown, the background appeared a light blue or light yellow, which was easy to distinguish. No positive labeling was seen in any of the negative control sections. The density changes of four types of endocrine cells during hibernation were shown in Table 2.

### 3.1. 5-HT-IR cells

5-HT-IR cells were distributed throughout the entire digestive tract, which were most predominant in duodenum and jejunum. During hibernation, the distributed density increased significantly in cardia, fundus, pylorus and duodenum ($P < 0.05$), did not change significantly in other parts ($P > 0.05$). The IR cells in esophagus were pyramid or spindle in shape, which situated in the basal portions of the epithelial mucosa, few IR cells with cytoplasmic processes were visible in esophagus (Fig. 1). During hibernation, 5-HT-IR cells were round or pyramid in shape, no cells with cytoplasmic processes were visible in esophagus (Fig. 2). The IR cells in stomach and small

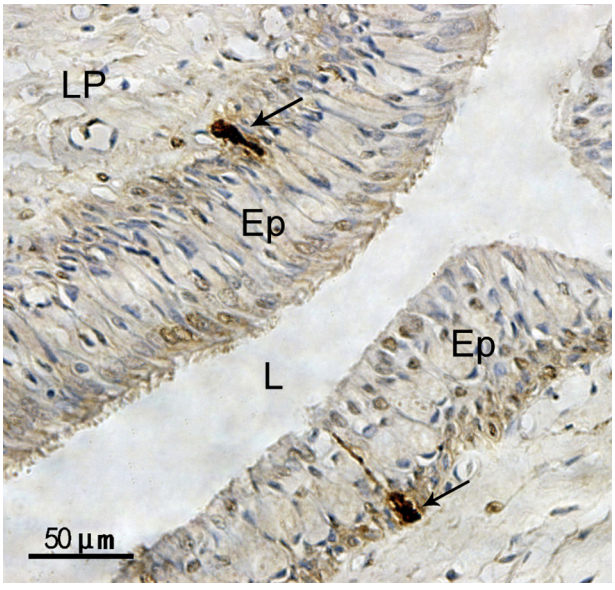


**Fig. 1.** 5-HT-IR cells in esophagus during active period, SABC method. Arrows: point to IR cells; L: lumen of digestive tract; Ep: epithelium; GG: gastric gland; LP: lamina propria.