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An immunohistochemical study on the gastro-entero-pancreatic endocrine cells of the cat-shark, *Scyliorhinus torazame*

Hyeung-sik Lee, Jae-hyun Lee*

Department of Biology, Kyungsan University
*College of Veterinary Medicine, Kyungpook National University**

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두툽상어(*Scyliorhinus torazame*)의 위장관 내분비세포에 관한 면역조직화학적 연구

이형식 · 이재현*

경산대학교 생물학과
경북대학교 수의과대학*

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초록 : 두툽상어의 위장관 내분비세포의 부위별 분포, 출현빈도 및 세포의 종류를 밝히고자 면역조직화학적 방법으로 관찰하였던 바, 5종의 면역반응세포가 동정되었다.

5-HT 면역반응세포는 최고의 빈도로, somatostatin 면역반응세포는 소수의 위부위와 극소수의 장부위에 걸쳐 각각 전장관에서 관찰되었다. Glucagon과 BPP 면역반응세포는 십이지장과 직장을 제외하고 다양한 빈도로 소화관 전체에서 출현하였다. 한편 Gas/CCK 면역반응세포는 소장부위에서 국한하여 소수 분포하였다.

이상에서 두툽상어의 위장관 내분비세포는 부위별 분포에 있어서 다른 종과 유사하였으나, 출현빈도는 다소 낮게 나타났음을 알 수 있었다.

Key words : cat-shark, gastrointestinal tract(GIT), endocrine cell, immunoreactive cell

Introduction

The gastrointestinal tract(GIT) of cartilaginous fishes is characterized by having the ileum with spiral valves, which compensates for a short intestine.

In recent years have been done on the intensive studies in the pylogeny of the endocrine cells in the GIT and a valuable of information has been accumulated on various vertebrates.

However, little work has known about endocrine cells of cartilaginous fishes using electron microscopes[1-5] and immunohistochemistry[6-11]. Furthermore, the cat-shark, a kind of the elasmobranch is completely unknown the presence of endocrine cells in the GIT except for those reported on the duodenum[1], rectum[3] and the pancreas[12].

The purpose of the present study was to clarify the

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Address reprint requests to Dr Hyeung-sik Lee Department of Biology, Kyungsan University, Kyungsan 712-240, Republic of Korea.

occurrence and frequency of each endocrine cells in the GIT of the cat-shark, *Scyliorhinus torazame, by specific* immunohistochemistry.

Materials and Methods

Five adult specimens of both sexes of the cat-shark, *Scyliorhinus torazame*, were used in this study. Samples from 8 portions of the GIT(Fig 1) were fixed in Bouin's fluid. After paraffin embedding, 4μm histological sections were prepared. The presentative sections were then deparaffinized, rehydrated and immunostained with peroxidase antiperoxidase(PAP) method[13]. Background blocking was performed with normal goat serum prior to incubation with specific antiserum(Table 1). After rinsing in PBS buffer, the sections were incubated in secondary serum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in HCl Buffer. After immunostaining, the sec-

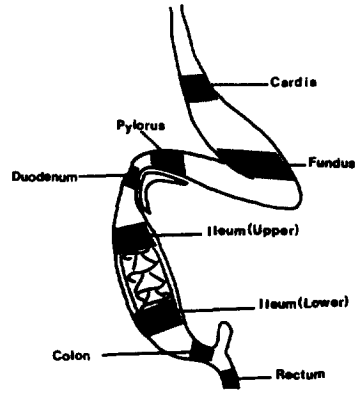

Fig 1. Sampling portions in the gastrointestinal tract of the catshark. a. cardia, b. fundus, c. pylorus, d. duodeunm, e. upper ileum, f. lower ileum, g. colon, h. rectum.

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
5-hydroxytryptamine (5-HT)	8535028	Immunonuclear Corp.	1 : 10,000
Glucagon	8635013	〃	1 : 800
Insulin	8622014	〃	1 : 2,000
Bovine chromogranin (BCG)	8541011	〃	1 : 500
Porcine chromogranin (PCG)	8541012	〃	1 : 2,000
Gastrin/cholecystokinin (Gas/CCK)	i600/400	Union Chimique Belge, bioproducts	1 : 100
Bovine pancreatic polypeptide (BPP)	i607	〃	1 : 5,000
Somatostatin	CA325	Cambridge Research Biochemical Ltd.	1 : 1,000

* All antisera were raised in rabbits except for insulin which was raised in a guinea pig.

tions were lightly counterstained with Mayer's hematoxylin.

Results

By means of immunohistochemical method five endocrine cell types were observed viz. 5-HT-, glucagon-, Gas/CCK-, BPP- and somatostatin-immunoreactive cells. The regional distribution and the relative frequency of these cell types in the various parts of the GIT are shown in the Table 2.

In the stomach the immunoreactive cells occurred in the tubular glands, whereas in the intestine they were located mostly in the columnar epithelia. On the other hand, immunoreactive cells generally were tall

Table 2. The regional distribution and relative frequency of immunoreactive cells in the GIT of the cat-shark, *Scyliorhinus torazame*

	Stomach			Duodenum	Ileum		Colon	Rectum
	Cardia	Fundus	Pylorus		Upper	Lower		
5-HT	+	++	+++	++	+	+	++	++
Glucagon	+	+	+	-	±	+	±	-
Insulin	-	-	-	-	-	-	-	-
BCG	-	-	-	-	-	-	-	-
PCG	-	-	-	-	-	-	-	-
Gas/CCK	-	-	-	+	+	+	-	-
BPP	+	±	+++	-	+	++	±	-
Somatostatin	+	+	+	±	±	±	±	±

- not detected, ± rare, + a few, ++ moderate, +++ numerous

and slender type which they protruded a fine process from the basal lamina to the gut lumen, except for glucagon-, BPP- and somatostatin-immunoreactive cells in the stomach.

5-HT-immunoreactive cells, reaching a peak in the pyloric region were demonstrated throughout the GIT. In the cardiac and the fundic gland regions, the distributions of these cells were scattered mainly far from the basal portion of the epithelia(Figs 2a-c), whereas in the pyloric gland region they were located to the basal portions of the epithelia(Fig 2d). In the intestines, the majority of these cells were inserted between the columnar cells of mucosa, having long cytoplasmic processes(Figs 2e-i).

Glucagon-immunoreactive cells were distributed in the epithelia throughout the GIT with the exception of the duodenum and the rectum. In the stomach, a few numbers of these cells were interspersed in the tubular gland regions(Figs 3a-c), whereas they were a few in numbers in the lower ileum(Fig 3e), and rare in the upper ileum(Fig 3d) and the colon(Fig 3f). In addition these cells were identified mostly in the columnar cells(Figs 3d-f).

A few numbers of Gas/CCK-immunoreactive cells were detected only in the duodenum and the ileum (Figs 4b, c). They were a slender columnar shape.

BPP-immunoreactive cells distributed predominantly in the pyloric gland regions were found in the

overall GIT except for the duodenum and the rectum. While these cells were observed beneth the mucosal epithelia in the cardiac and the fundic gland regions (Figs 5a, b), in the pyloric gland regions they were detected mainly in the basal portion of glands(Fig 5c).

Somatostatin-immunoreactive cells were distributed the whole GIT. In the stomach(Figs 6a-e), a few numbers of these cells were detected in the basal tubular glands. Also, they were rare in the intestinal mucosal epithelia with almost uniform frequency(Figs 6f-j).

Discussion

In the present study, five kinds of immunoreactive cells were demonstraed in the GIT of the cat-shark by specific immunohistochemical method. However, insulin- and CGs-immunoreactive cells were not detected in the GIT of the cat-shark. Although insulin-immunoreactive cells were observed in the small and the large intestines of the dogfish[7], these cells could not be found in the other dogfish[9].

El-Salhy et al[14] reported that 5-HT-immunoreactive cells were found in the GIT in all the gnathostome species investigated. These cells have been reported to occur in the wider distribution and higher frequency than other endocrine cell types in the GIT[7,15,16]. We found that the relative number of the cells detected in the cardiac gland regions and the ileum were less frequent than those of the other regions. This distributional pattern was different from those in the other species[6,7,14] reported a concentration of 5-HT-immunoreactive cells in the distal intestine.

El-Salhy[7] reported that while glucagon-immunoreactive cells distributed in the whole GIT of the spiny dogfish, the rectal mucosa were devoid of these cells in the dogfish[9] and the ratfish[10]. We observed that these cells were demonstrated throughout GIT except for the duodenum and the rectum, whereas the regional relative frequency in these cells was greater in the stomach than in the intestine. These results were somewhat different from those reported previously[7,9,10].

A few number of Gas/CCK-immunoreactive cells were only restricted to the small intestine in the present study. Although similar findings have been reported on the spiny dogfish[6], dogfish[7] and the ratfish[10], Cimini et al[11] reported that these cells were found numerous in the entire GIT in the dogfish.

We demonstrated that BPP-immunoreactive cells were dominant only in the pylorus of the stomach and found in the entire GIT with the exception of the duodenum and the rectum. The pattern of regional distribution of these cells was quite different from that of the dogfish small intestine[7], but the cat-shark showed a similar pattern to that reported earlier[9]. These results also maybe reflect the species differences at the molecular level.

Somatostatin-immunoreactive cells were demonstrated a few in the stmach and rare in the intestine with almost uniform frequency. In the present study, these results were correlated well with the those reported previously[7,9,10], whereas the pattern of regional distribution of these cells in the rectum was quite differnt from the absence of the dogfish[6].

These results suggested that the difference of regional distribution of endocrine cells in the GIT of the cat-shark may be due to difference in molecular structure between the species and the antiserum rather than to the absence of those peptides. In the future, hybridizatin will be employed to demonstrate the site of production of a peptide by localization of it's mRNA or gene source.

In conclusion, we have thus demonstrated the characteristic patterns of distribution of five kinds of endocrine cells and their relative frequencies of the cat-shark.

Summary

The regional distribution and relative frequencies of gastrointestinal endocrine cells were studied immunohistochemically in the GIT of the cat-shark. Five kinds of endocrine cells were identified in this study. Mostly these cells were of open types, except for glucagon-, BPP- and somatostatin-immunoreactive cells

in the stomach which seemed to be of closed type. 5-HT-immunoreactive cells were detected throughout the GIT, and were more freguent than of the other regions. Glucagon-and BPP-immunoreactive cells were demonstrated in the entire GIT except for the duodenum an the rectum, and occurred in various frequencies. A few numbers of Gas/CCK-immunoreactive cells were restricted to the duodenum. Somatostatin-immunoreactive cells were distributed in the whole GIT, and were a few in numbers in the stomach and rare in the intestine, respectly. These results suggest that the pattern of the regional distribution is rather similar to that reported for previously, but relative number was less frequent than that of other species.

Legends for figures

Fig 2. 5-HT-immunoreactive cells throughout the GIT.
a, b. cardia, c. fundus, d. pylorus, e. duodenum, f. upper ileum, g. lower ileum, h. colon, i. rectum.
a, c-i; ×240, b; ×540.

Fig 3. Glucagon-immunoreactive cells throughout GIT with the exception of the duodenum and rectum.
a. cardia, b. fundus, c. pylorus, d. upper ileum, e. lower ileum, f. colon.
a-c, e: ×240, d, f; ×540.

Fig 4. Gas/CCK-immunoreactive cells in the duodenum(a), the upper(b) and the lower ileum(c). a-c; ×240.

Fig 5. BPP-immunoreactive cells in the GIT.
a. cardia, b. fundus, c. pylorus, d. upper ileum, e. lower ileum, f. colon, a-c, e; ×240, d, f; ×540.

Fig 6. Somatostatin-immunoreactive cells the entire GIT.
a-c. cardia, d. fundus, e. pylorus, f. duodenum, g. upper ileum, h. lower ileum, i. colon, j. rectum.
a, d-j; ×240, c; ×540.

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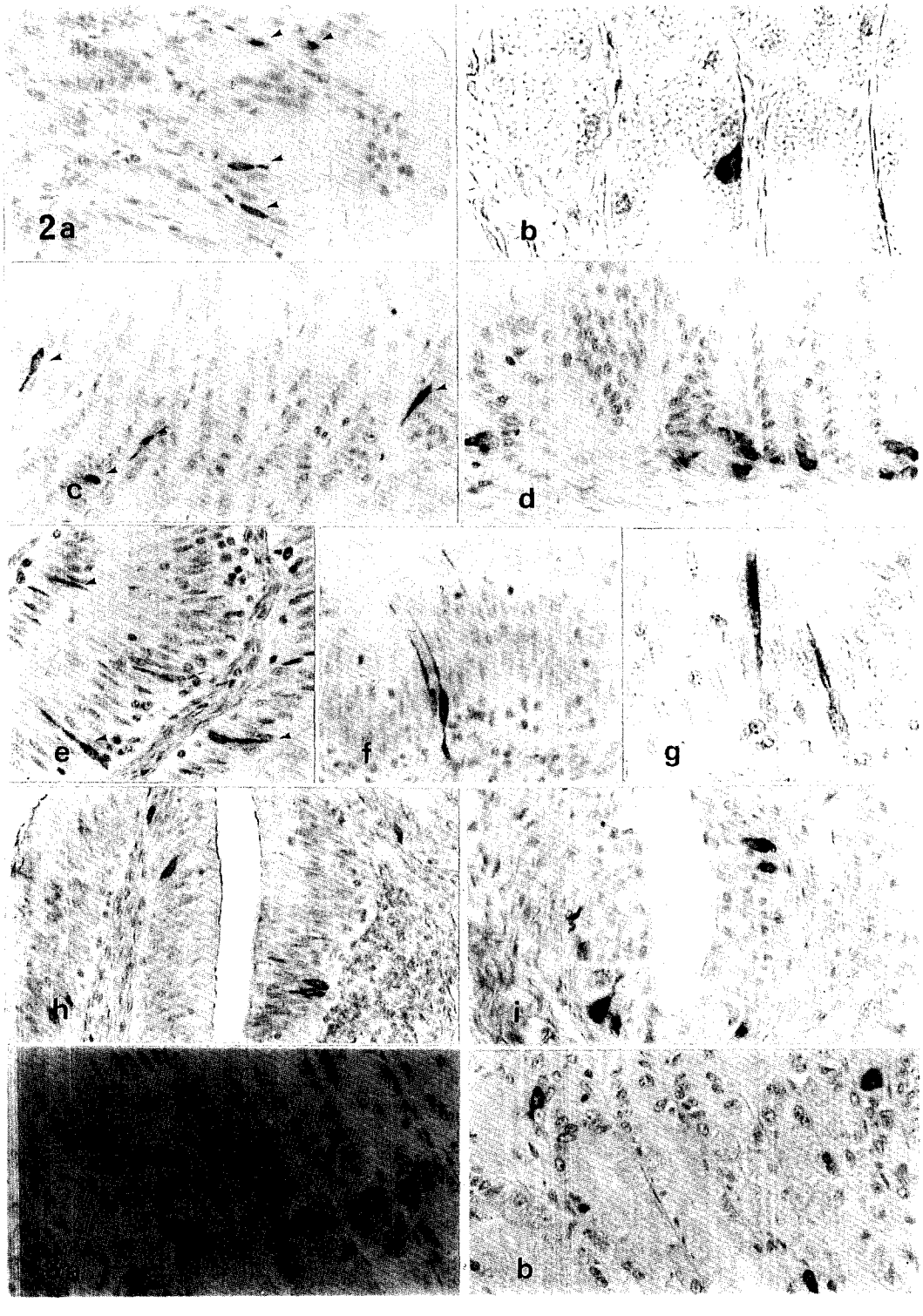
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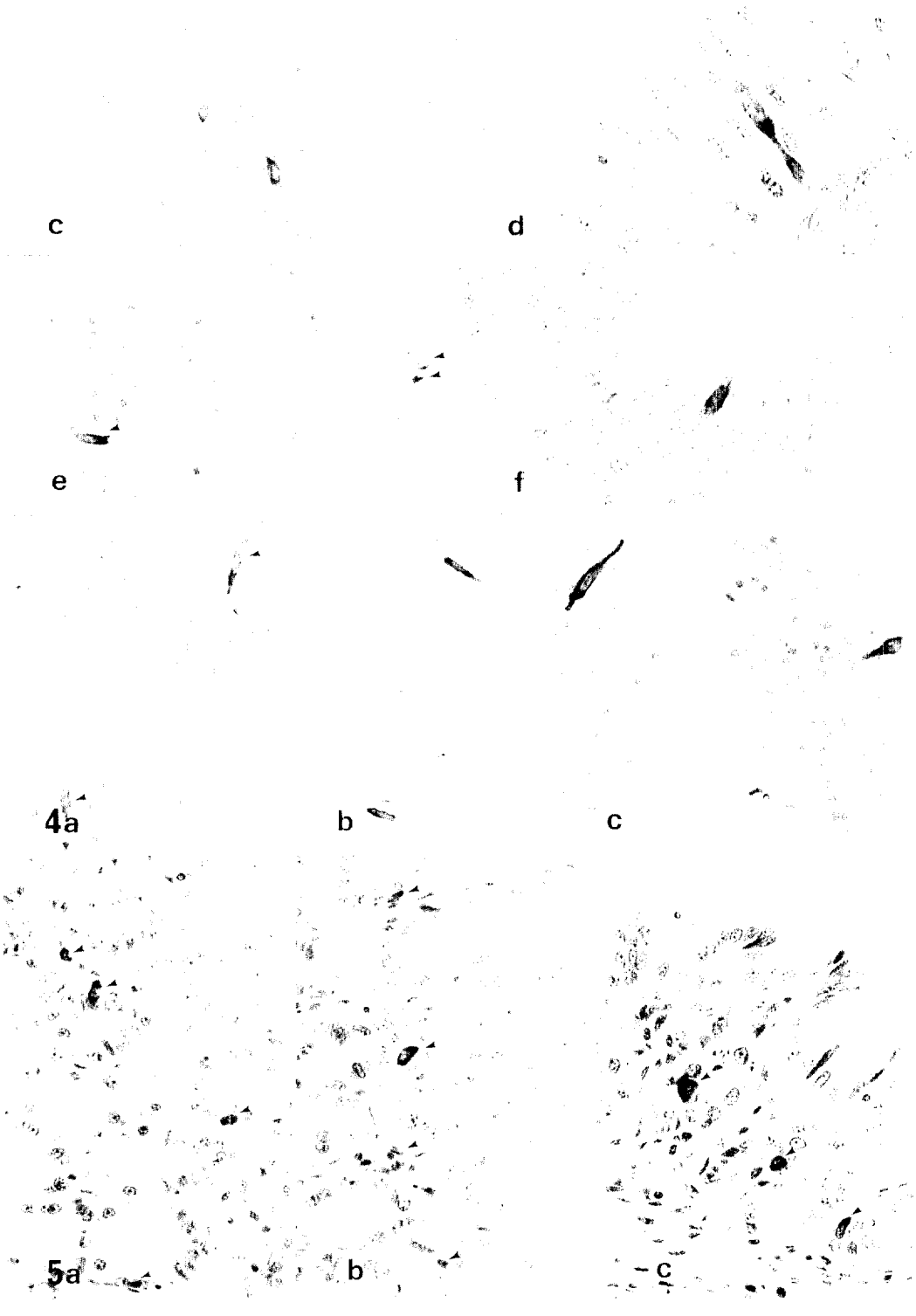
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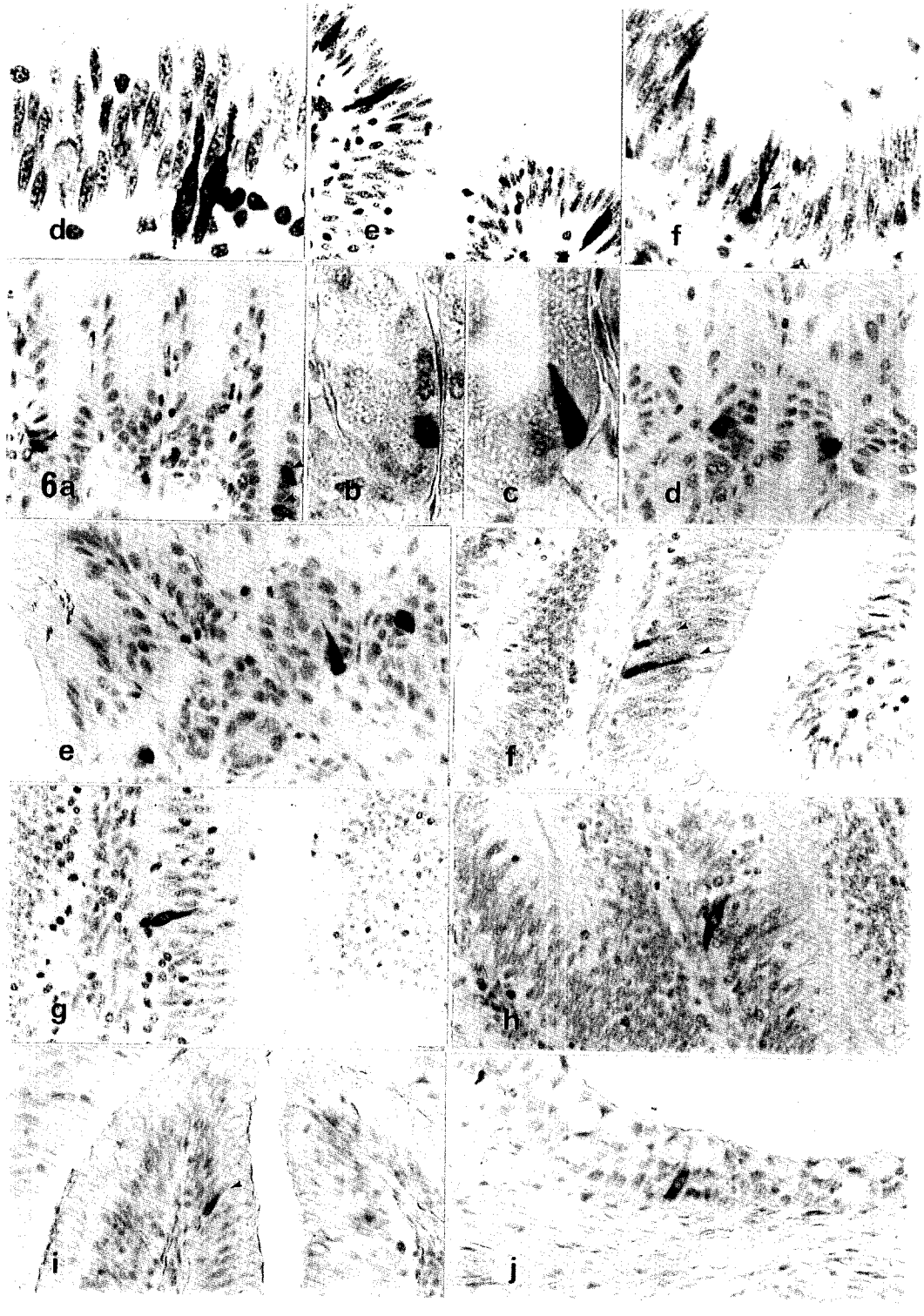
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