大韓獸醫學會誌(2000) 第40卷 第1號
Korean J Vet Res(2000) 40(1) : 17~26

An immunohistochemical study of endocrine cells in the alimentary tract and pancreas of the toad, *Bufo bufo gargarizans* Cantor

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(Received Dec 28, 1999)

두꺼비(*Bufo bufo gargarizans* Cantor)에서 위장췌내분비세포의 면역조직화학적 연구

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(1999년 12월 28일 접수)

Abstract : The regional distribution and relative frequencies of endocrine cells were studied immunohistochemically (PAP methods) in the alimentary tract and pancreas of the toad, *Bufo bufo gargarizans* Cantor using specific antisera against bovine Sp-1/chromogranin (BCG), serotonin, bombesin, gastrin, substance P (SP), somatostatin, insulin, glucagon, pancreatic polypeptide (PP), vasoactive intestinal polypeptide (VIP) and secretin. Nine kinds of endocrine cells were identified in this study. Spherical or spindleshaped immunoreactive (IR) cells were located in the gastric glands of stomach regions, in the basal portion of the epithelium of intestinal tract or esophagus, and in the exocrine or pancreatic islets with variable frequencies.

In the alimentary tract, BCG-IR cells were found in the fundus and pylorus with rare and a few frequencies, respectively. Serotonin-IR cells were demonstrated in the whole alimentary tract including the esophagus. Bombesin- and SP-IR cells were restricted to the stomach regions and gastrin-IR cells were restricted to the pylorus. Somatostatin-IR cells were detected throughout the whole alimentary tract except for the large intestine, However, insulin-, glucagon-, PP-, VIP- and secretin-IR cells were not detected in the alimentary tract.

In the pancreas of toad, the distribution and relative frequency of endocrine cells were similar

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to those of other mammals. Insulin-IR cells were located in the central portion of the pancreatic islets and interspaces of exocrine portions, and glucagon-, somatostatin- and PP-IR cells were detected in the marginal regions of the pancreatic islets and interspaces of exocrine. However, other IR cells were not found in the pancreas.

In conclusion, the regional distribution and relative frequency of the endocrine cells in the alimentary tract and pancreas of the toad were similar to other anuran species but some differences which might be caused by feeding habits and species specification were also observed.

Key words : endocrine cells, alimentary tract, toad, pancreas, immunohistochemistry

Introduction

Toad, *Bufo bufo gargarizans* Cantor, belonged to the order Anura have been widely distributed in Korea. Gastrointestinal endocrine cells dispersed in the epithelia and mucosal glands of the alimentary tract synthesized various kinds of gastrointestinal hormones and play and 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].

Many gastroenteropancreatic (GEP) neuropeptides have been isolated from the amphibian skin[4,5]. The GEP endocrine cells in the various amphibians have been extensively studied by histochemical[6], electron microscopical[7,8] and immunohistochemical[9] methods. Although about 17 types of endocrine cells including serotonin, somatostatin, glucagon, cholecystokinin (CCK)-8, chromogranin, pancreatic polypeptide (PP), bombesin, neurotensin, gastrin-releasing peptide, substance P (SP), polypeptide YY, secretin, gastrin, vasoactive intestinal polypeptide (VIP), motilin, met-enkephalin and β-enkephalin etc, have been detected in *Rana dybowskii*[10], *Rana pipens*[11], *Xenopus laevis*[11-13], *Rana esculenta*[14], *Bufo regularis*[15,16], *Rana catesbeiana*[17,18], *Rana nigromaculata*[19] and 8 species of anuran amphibian[9], there are not many works on the endocrine cells of the toad. In addition, Choi *et al*[20] reported the localization of endocrine cells in the alimentary tracts of six frog species habited in Korea. The present study aims to characterized the regional distributions and the relative frequencies of the endocrine cells in the alimentary tract and pancreas of the toad, *Bufo bufo gargarizans* Cantor, were investigated by immunohistochemical methods using specific antisera against bovine Sp-1/chromogranin (BCG), serotonin, bombesin, gastrin, SP, somatostatin, insulin, glucagon, PP, VIP, and secretin.

Materials and Methods

Five adult toads, *Bufo bufo gargarizans* Cantor, were captured in Kyungsan, Korea without sexual distinction and used in this study. Samples from seven portions of the alimentary tract (esophagus, fundus, pylorus, duodenum, small intestine, large intestine and pancreas) were fixed in Bouin's solution. After paraffin embedding, 3~4μm serial sections were prepared with routine methods. The each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase anti-peroxidase (PAP) method[21]. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsing in phosphate buffered saline (PBS; 0.01M, pH 7.4), the sections were incubated in secondary antiserum. 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 Tris-HCl buffer (0.05M,

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Dilution
BCG[1)]	517210	Incstar Corp., Stillwater	1 : 1,000
Serotonin	BO68082C	BioGenex Lab., San Ramon	1 : 20
Bombesin	8652015	Immunonuclear Corp., Stillwater	1 : 1,000
Gastrin	PUO190796	BioGenex Lab., San Ramon.	1 : 20
Substance P	B9C 35	Sera Lab., Sussex	1 : 1,000
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
Insulin	PUO290395	BioGenex Lab., San Ramon.	1 : 24
Glucagon	PUO390598	BioGenex Lab., San Ramon.	1 : 20
PP[1)]	PUO660495	BioGenex Lab., San Ramon.	1 : 20
VIP[1)]	B95C	Sera Lab., Sussex	1 : 1,000
Secretin	BO67122A	BioGenex Lab., San Ramon.	1 : 20

* All antisera were raised in rabbits except for insulin, which was raised in guinea pigs.
1) BCG : bovine Sp-1/chromogranin, PP : pancreatic polypeptide, VIP : vasoactive intestinal polypeptide.

pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's hematoxylin and the immunoreactive cells were observed under light microscope.

Result

In this study, six kinds of the IR cells were detected with the antisera against BCG, serotonin, bombesin, gastrin, SP and somatostatin in the alimentary tract and four kinds of the IR cells were demonstrated with antisera against insulin, glucagon and PP in the pancreas. According to the location of the alimentary tract and pancreas, different distributions and relative frequencies of these IR cells were observed. These differences are shown in Table 2. However, no VIP- and secretin-IR cells were detected in this study.

Sphericalshaped BCG-IR cells were restricted to the gastric gland of fundus (Fig 1a) and the pylorus (Fig 1b) with rare and a few frequencies respectively.

Serotonin-IR cells were demonstrated in the basal portions or interspaces of the epithelia of the whole alimentary tract except for the stomach regions where these cells were located in the gastric gland regions with spindle to spherical-shaped. A few serotonin-immunoreactive cells were detected in the esophagus (Fig 2a) but numerous or a few in the stomach regions (Fig 2b and c). These IR cells were most predominant in the pylorus but only a few IR cells were found in the other intestinal regions (Fig 2d and e).

Bombesin-IR cells were restricted to the gastric glands of fundus with rare frequency (Fig 3a) and the pylorus with a few frequency (Fig 3b) with spherical shape. These results were similar to that of BCG-IR cells. And spherical shaped gastrin-IR cells were also restricted to the gastric glands of pylorus with moderated frequency (Fig 4).

The regional distribution and shape of SP-IR cells were similar to those of BCG-, bombesin- and gastrin-IR cells. SP-IR cells were detected in the stomach with a few frequencies (Fig 5a and b).

Spherical to spindle shaped somatostatin-IR cells were observed in the basal portion of the epithelia of the whole alimentary tract except for the rectum and the stomach regions. In the stomach regions, somatostatin-IR cells were resembled to those of serotonin-IR cells but they were not demonstrated in the rectum. The relative frequencies of somatostatin-IR cells were rare in the esophagus (Fig 6a),

Table 2. Regional distributions and relative frequencies of the endocrine cells in the alimentary tract of the toad, *Bufo bufo gargarizans* Cantor

	Esophagus	Fundus	Pylorus	Duodenum	Small intestine	Large intestine	Pancreas
BCG[1)]	-*	±	+	-	-	-	-
Serotonin	±	+	+++	±	±	±	-
Bombesin	-	±	+	-	-	-	-
Gastrin	-	-	++	-	-	-	-
Substance P	-	+	-	-	-	-	-
Somatostatin	±	±	+-+	±	±	-	++-
Insulin	-	-	-	-	-	-	+++
Glucagon	-	-	-	-	-	-	+-+
PP[1)]	-	-	-	-	-	-	++
VIP[1)]	-	-	-	-	-	-	-
Secretin	-	-	-	-	-	-	-

[1)] BCG : bovine Sp-1/chromogranin, PP : pancreatic polypeptide, VIP : vasoactive intestinal polypeptide
* Relative frequencies ; +++ : numerous, ++ : moderate, + : a few, ± : rare, - : not detected.

fundus (Fig 6b), duodenum (Fig 6d) and small intestine (Fig 6e and f). In the pylorus, somatostatin-IR cells were most predominantly detected with numerous frequency (Fig 6c). In the pancreas, somatostatin-IR cells were numerously detected in interspaces of exocrine portion and marginal regions of the pancreatic islets (Fig 7c).

Insulin-IR cells were numerously found in the central regions of pancreatic islets and interspaces of the exocrine portions but not detected in the alimentary tract (Fig 7a).

Glucagon- and PP-IR cells were detected in the regions, which were similar to somatostatin-IR cells in the pancreas, but they were not demonstrated in the alimentary tract. Glucagon-IR cells were detected with numerous frequency (Fig 7b) and PP-IR cells were located with moderate frequency (Fig 7d).

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 alimentary tract[22,23].

Chromogranins have been found to occur in large variety of endocrine organs and cells outsice the adrenal medulla, and they have been claimed as common markers of all neuroendocrine cells[24,25]. However, demonstrated chromogranin-IR cells were restricted to the stomach regions in *Xenopus laevis* [12], *Rana nigromaculata* [19] and in the present study. In addition, it is reported that the relative frequencies in *Rana dybowskii* [10] were smaller than other typed endocrine cell. These results suggested that chromogranin was not suitable as a marker of other endocrine cells in the anuran species.

Serotonin consists of monoamines and is widely distributed in nervous system and GEP endocrine cells[26]. El-Salhy *et al* [26] reported that serotonin-IR cells found throughout the gastrointestinal tract (GIT) of all species and established in the alimentary tract at the early stage of vertebrate evolution. The regional distributions and relative frequencies of serotonin-IR cells were detected in the whole GIT of *Rana dybowskii* [10], *Xenopus laevis* [12], *Rana nigromaculata* [19] and *Rana catesbeiana*[18]. In addition, Choi *et al* [20] reported the localization of serotonin-IR cells in the alimentary tracts of six frog

species habited in Korea including the tree frog. According to these previous reports, these cells were most predominant in the pylorus except for *Rana nigromaculata* [19] that were most predominant in the fundus and duodenum. It is reported that this variance of the relative frequencies in the anuran species might depend on sampling time or season[10]. In the present study, these cells were observed in the whole alimentary tract like the previous reports.

Like the previous studies[11,15,17,19], the present study showed that bombesin-IR cells restricted to the fundus and pylorus, and secretin-IR cells were not found. These findings are similar to Lee *et al*[9], Buchan[19] and Van Noorden and Falkmer[27] who reported that these IR cells were not detected in the lower vertebrates. However, El-Salhy *et al*[15] reported that secretin-IR cells were demonstrated in the whole GIT of *Bufo regularis* except for the rectum. It is reported that PP-IR cells were found in the stomach and small intestine of the anuran species[15,20,28]. Differing from these reports, PP-IR cells were not detected in this study.

It is generally accepted that gastrin and CCK-8 originated from same ancestor and in the human duodenum a large fraction of these cells, besides reacting with non-C terminal CCK antibodies and C-terminal gastrin/CCK antibodies, also showed immunoreactivity with C-terminal gastrin-34 antibodies, colocalised with CCK in a variable portion of secretory granules[29]. In the anuran species, gastrin-, CCK-8- or gastrin/CCK-IR cells were restricted to the pylorus, duodenum and ileum[9,10,12,20]. In the present study, different from those of other anuran species, gastrin-IR cells were restricted to the pylorus.

SP has been found to be colocalised with serotonin and chromogranin A in the argentaffin granules of a subpopulation of gut EC cells, called EC_1 cells[29]. They has been found in the gut, adrenal medulla and some pheochromocytomas are well as in some paraganglionic cells of the carotid body and cervical, celiac and mesenteric ganglia [29]. It is difficult to find the reports dealing the regional distribution and relative frequency of these IR cells in the anuran species except for immunoreactivity in the gastrointestinal nerve system including myenteric and submucosal plexus[31]. Anyway, these IR cells were restricted to the stomach in this study.

Somatostatin, which consists of 14 amino acics, was isolated from hypothalamus of sheep for the first and it could be subdivided into straight form and cyclic form[32]. It is well known that somatostatin-IR cells show the widest distribution in the whole GIT of all vertebrate species investigated, including the primitive agnathans with serotonin-IR cells[33]. In the anuran species, these cells were detected in *Rana dybowskii*[10], *Rana esculenta*[14], *Xenopus laevis*[12], *Bufo regularis*[15], *Rana nigromaculata*[19], *Rana catesbeiana* [18], 8 species of Anura[9] and six frog species habited in Korea including the tree frog[20]. According to these reports, somatostatin-IR cells were most predominant in the fundus but decreased distally along the GIT except for tree frog in which they were most predominant in the pylorus and thereafter decreased toward to the distal portion of intestine and finally not detected in the rectum[20]. In the present study, somatostatin-IR cells were detected throughtout the whole alimentary tract except for the rectum and the regional distributions are agreed with those of the previous studies[8,10,12,14,15,18-20]. However, the relative frecuencies are somewhat lower than those of other reports[8,10,12,14,15,18-20].

VIP is a 28 amino acid peptide, which was originally isolated from porcine intestine and recognized for its potent vasodilatory effect[34]. Immunoreactivity of VIP in intestinal nerve was detected in seven species of anuran species using immunohistochemical and radioimmanological techiques[35]. In this report, the regional distribution and relative frequency of VIP-IR cells showed species-specific differences and Larsson *et al*[36] reported that the distribution of VIP secretory endocrine cells demonstrated by the different VIP antisera were varied. However, no VIP-IR cells were found in this study.

According to the anuran species, variable distributional patterns of glucagon-IR cells in the GIT were reported[10,12,15,19]. Especially, Lee and Lee[12] reported that these cells were restricted to the fundus of *Xenopus laevis*. However, El-Salhy *et al*[15] and Lee *et al*[19] reported that they were found in the whole GIT of *Bufo regularis* and *Rana nigromaculata* except for rectum. Differing from previous reports[10,12,15,19], these cells were not observed in the present study. It is re-

ported that PP-IR cells were found in the stomach and small intestine of the anuran species[15,28]. The present study did not found these cells either.

The locations and relative frequencies of insulin-, glucagon-, somatostatin- and PP-IR cells in the pancreas of anuran species have been accepted that insulin-IR cells were located in the central portion of the pancreatic islets and interspaces of the exocrine portions and glucagon-, somatostatin- and PP-IR cells were found in the marginal zone of the pancreatic islets and interspaces of the exocrine portion[37], and it is well coincidence with the results of the present study.

In conclusion, the regional distributions and relative frequencies of the endocrine cells in the alimentary tract and pancreas of the toad were resembled to the other anuran species except for some differences which might be caused by feeding habits and species specification.

Legends for figures

Fig 1. BCG-IR cells in the alimentary tract of the toad.
a. Fundus b. Pylorus
a, b : × 240, PAP methods

Fig 2. Serotonin-IR cells in the alimentary tract of the toad.
a. Esophagus b. Fundus c. Pylorus d. Duodenum e. Large intestine
a, d, e : × 480 ; b, c : × 240, PAP methods

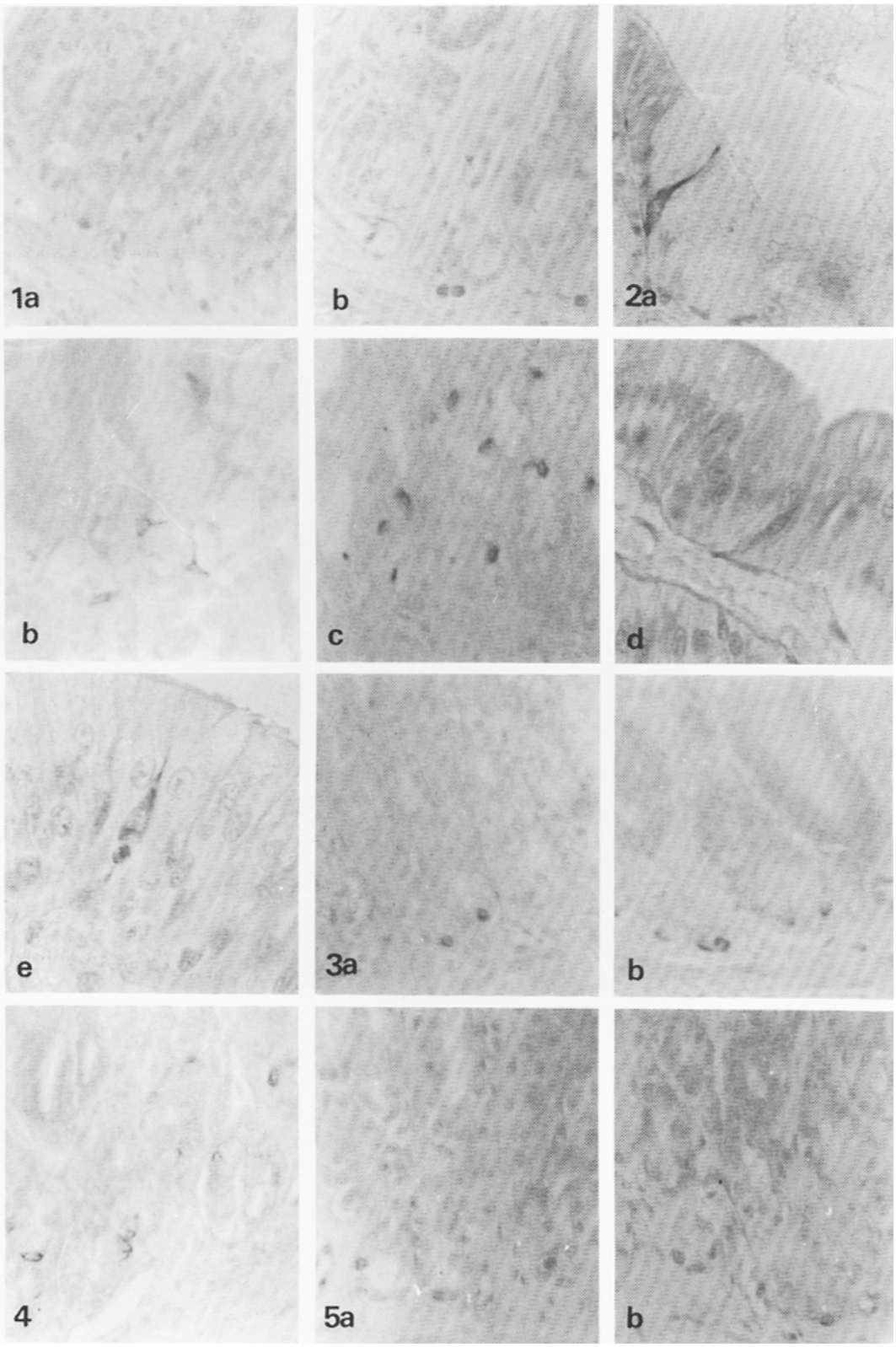
Fig 3. Bombesin-IR cells in the alimentary tract of the toad.
a. Fundus b. Pylorus
a, b : × 240, PAP methods

Fig 4. Gastrin-IR cells in pylorus of the toad. × 240, PAP methods

Fig 5. SP-IR cells in the alimentary tract of the toad.
a. Fundus b. Pylorus
a, b : × 240

Fig 6. Somatostatin-IR cells in the alimentary tract of the toad.
a. Esophagus b. Fundus c. Pylorus d. Duodenum e, f. small intestine
a, d-f : × 480 ; b, c : × 240, PAP methods

Fig 7. IR cells in the pancreas of the toad.
a. Insulin-IR cells b. Glucagon-IR cells c. Somatostatin-IR cells d. PP-IR cells
a-d : × 240, PAP methods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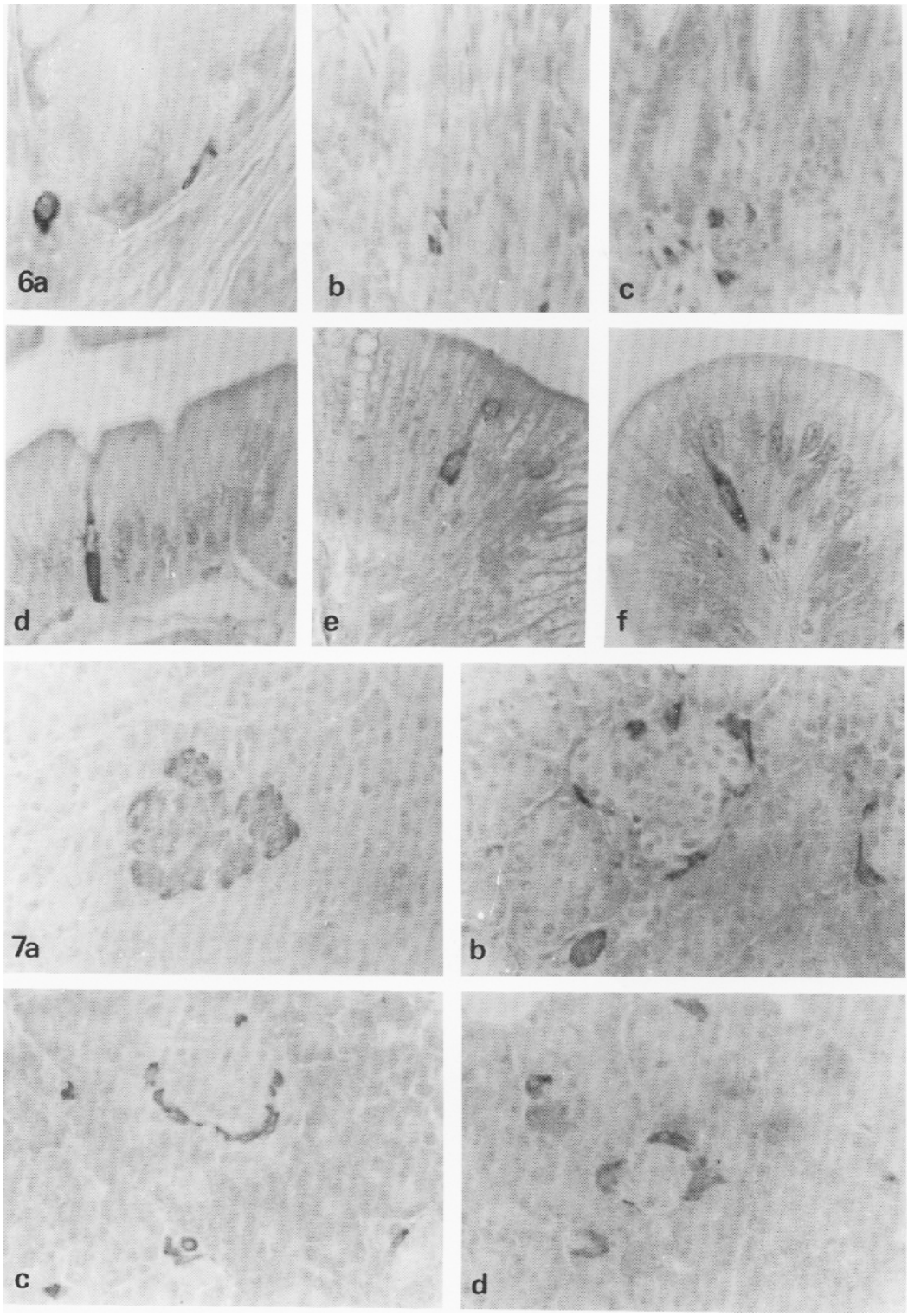
6a
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