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An immunohistochemical study of the endocrine cells in gastrointestinal tract of the Korean native goat

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한국 재래산양의 위장관에 출현하는 내분비세포의 면역조직화학적 연구

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초록 : 한국 재래산양의 위장관 내분비세포를 면역조직화학적으로 관찰하였던 바 5-HT, somatostatin, Gas/CCK, glucagon, chromogranin, PP 면역반응세포들이 동정되었다. 한국 재래산양의 위장관에 있어서 이들 면역반응세포의 부위별 분포와 출현빈도의 특징은 다음과 같다.

5-HT 면역반응세포는 제 4위 및 유문부에서 보다 대장과 소장에서 더 많이 출현하였다. Somatostatin 면역반응세포는 대장과 소장에서 보다 제 4위와 유문부에서 다수 출현하였다. Gas/CCK 면역반응세포는 유문부에서 가장 다수로 분포하였으며 기타 부위에서는 소수로 출현하였다. 대장과 소장에서는 중등도의 glucagon 면역반응세포가 관찰되었으나, 제 4위와 유문부에서는 소수로 관찰되었다. Chromogranin 면역반응세포는 전 위장관에서 골고루 그리고 아주 많이 출현하였다. PP 면역반응세포는 대장에서 중등도로, 회장에서 소수로 분포하였다. Insulin 면역반응세포는 전 위장관에서 관찰할 수 없었다.

Key words: immunoreactive cell, gastrointestinal tract, Korean native goat, pyloric region, intestine.

Introduction

Though gastrointestinal regulatory peptides and amines in various mammalia have been intensively studied,[1~8] immunohistochemical studies on the ruminants are very scarce.[9~11] There has been only one investigation of the gastrointestinal endocrine cells of the Korean native goat using immnunohistochemical technique.[12]

The purpose of the present study was to determine the distribution and frequency of immunoreactive endocrine cells in the gastrointestinal tract of the Korean native goat using seven specific antisera (somatostatin, gastrin / cholecystokinin, glucagon, serotonin, pancreatic polypeptide, insulin, chromogranin) for gastro-entero-pancreatic hormones.

Materials and Methods

The adult Korean native goats of both sexes were used in this study. Seven regions of the gastrointestinal tract were dissected out and fixed with Bouin's fluid or 10% buffered formalin. After paraffin embedding, serial sections were cut at 4 or 6μm thickness. Representative deparaffinized and rehydrated sections

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Table 1. Antisera used

Antisera raised[a]	code	source	dilution
Somatostatin	CA325	Cambridge Research Biochemical (CRB), Billerica	1 : 1,000
Gastrin/ Cholecystokinin	i600/004	Union Chimique Belge (UCB)-bioproducts	1 : 100
Pancreatic polypeptide	i607	UCB-bioproducts	1 : 5,000
Glucagon	8635013	Immuno Nuclear Corp. (INC), Stillwater	1 : 800
5-Hydroxytryptamine	8535028	INC	1 : 10,000
Insulin	8622014	INC	1 : 2,000
Chromogranin	8541012	INC	1 : 2,000

a) All antisera were raised in the rabbits, except that against insulin, which was raised in a guinea pig.

Table 2. Distribution and relative frequency of the gastrointestinal endocrine cells in the Korean native goat

	Abdomen stomach		Small intestine			Large intestine	
	Fundic	Pyloric	Duodenum	Jejunum	Ileum	Colon	Rectum
Serotonin	++	++	+++	+++	+++	+++	+++
Somatostatin	+++	+++	++	+	+	++	++
Gas/CCK	+	+++	++	+	+	+	+
Glucagon	+	+	+	++	++	++	++
Insulin	−	−	−	−	−	−	−
Chromogranin	+++	+++	+++	+++	+++	+++	+++
PP	−	−	−	−	+	++	++

−: absent, +: few, ++: moderate, +++: numerous, +++: very numerous. Gas/CCK: Gastrin/Cholecystokinin, PP: Pancreatic polypeptide.

were stained with hematoxylin-eosin or periodic acid Schiff(PAS) for histological examination.

The peroxidase-antiperoxidase(PAP) method[13] was used in this study to identify specific endocrine cells. Details of the antisera used are shown in the Table 1. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger[13] and included replacement of the specific antiserum with antiserum preincubated with corresponding antigen. The antisera and control sera were diluted in 0.01M phosphate buffer with 0.5M saline (pH 7.4) to prevent nonspecific binding of immunoglobulins by ionic interaction. Controls for all immunohistochemical reations were negative.

To prevent nonspecific staining, the sections were incubated with non-immunized goat serum prior to incubation with specific antisera. After staining, each section was lightly counterstained with Mayer's hematoxylin, dehydrated, cleared in xylene and mounted. The relative frequency of occurrence of each type of immunoreactive cells was allocated to one of five categories according to their frequency as seen by light microscope. The relative frequency and distribution of immunoreactive cells in the gastrointestinal tract are summarized in Table 2.

Results

In this study insulin-immunoreactive cells were not detected at any sites along the gastrointestinal tract of the Korean native goat.

In the fundic region, immunoreactive cells for serotonin(5-HT) (Fig 1c), somatostatin(Figs 1a, b),

gastrin/cholecystokinin(Gas/CCK), glucagon(Fig 1d) and chromogranin were found in the glands and epithelia. These endocrine cells were round or oval in shape, and the minority of endocrine cells were often established luminal contact by their apical cytoplasmic processes. Numerous chromogranin- and somatostatin-immunoreactive cells, moderate numbers of 5-HT-immunoreactive cells and a few glucagon-immunoreactive cells were distributed in the fundic glands. However, PP-immunoreactive cells were not found in the fundic region(Table 2).

In the pyloric region, 5-HT-, somatostatin-, Gas/CCK-, glucagon- and chromogranin-immunoreactive cells were distributed in the pyloric glands and epithelia(Table 2, Figs 2a-c, 3a-b, 4a-b). Very numerous somatostatin-and chromogranin-immunoreactive cells as well as moderate numbers of 5-HT- and a few glucagon-immunoreactive cells were found. These endocrine cells were pyramidal or oval in shape. The majority of endocrine cells in this region were established a contact with the lumen via slender, elongated, apical cytoplasmic processes. PP-immunoreactive cells were also not found in this region.

In the small intestine, 5-HT-, somatostatin-, Gas/CCK-, glucagon- and chromogranin- immunoreactive cells were distributed in the intestinal glands and epithelia(Table 2). These endocrine cells were pyramidal or elongated in shape. The majority of endocrine cells in this region were established contact with the lumen via an apical long cytoplasmic processes(Figs 5~7). In the duodenum, very numerous 5-HT-(Fig 5b) and chromogranin-(Fig 5e) immunoreactive cells were found, while moderate numbers of somatostatin-(Fig 5a) and Gas-CCK-(Fig 5c) immunoreactive cells were found. A few glucagon-immunoreactive cells were also found. Numerous 5-HT-(Fig 6b), chromogranin-(Fig 6e), moderate numbers of glucagon-(Fig 6d), and a few somatostatin-(Fig 6a) and Gas/CCK-(Fig 6c) immunoreactive cells were identified in the jejunum. In the ileum, numerous 5-HT-(Fig 7b), chromogranin-(Fig 7d), moderate numbers of glucagon-(Fig 7f), a few somatostatin-(Fig 7a) and Gas/CCK-(Fig 7c) immunoreactive cells were found. However, few PP-immunoreactive cells were detected in this region.

In the large intestine, cells immunoreactive for 5-HT, somatostatin, Gas/CCK, glucagon, chromogranin and PP were scattered within the intestinal epithelia and glands(Figs 8a-g, 9a-e). These endocrine cells were pyramidal or elongated in shape. The majority of enocrine cells in this region were also estabilshed contact with the lumen via an apical cytoplasmic processes. In the colon and rectum, numerous 5-HT-(Figs 8b, 9b), chromogranin-(Figs 8e, 9e), moderate numbers of somatostatin-(Figs 8a, 9a), glucagon-(Figs 8c, 9d) and PP-(Figs 8f, g) immunoreactive cells were found, but a few Gas/CCK-(Figs 8d, 9c) immunoreactive cells were identified(Table 2).

Discussion

Thirteen types of endocrine cells have been reported in the gastrointestinal tract of cow[11] and sheep.[9] Ohara et al.[14] reported twelve types of endocrine cells in Japanese field vole, one of rodents whose digestive system is most adapted to herbivorous eating habits. Recently, Cho and Kitamura[10] reported eleven types of endocrine cells immunoreactive for chromogranin, serotonin, somatostatin, glucagon, bovine pancreatic polypeptide, motilin, gastric inhibitory polypepetide, neurotensin, secretin, gastrin and substance P in the gut of the Korean native cattle. Four types of endocrine cells, somatostatin-, gastrin-, secretin- and glucagon-immunoreactive cells were also identified immunocytochemically in the gastrointestinal tract of the ruminants.[12]

In the present study, six types of endocrine cells immunoreactive for 5-HT, somatostatin, Gas/CCK, glucagon, chromogranin and PP were identified in the gastrointestinal tract of the Korean native goat.

5-HT-immunoreactive cells were remarkable in the duodenum and numerous in the other small and large intestines, but moderate numbers in the abdomen stomach. Generally, the regional distribution of 5-HT-immunoreactive cells in the Korean native goat is similar to that of other mammals.[4,10,11] However, these endocrine cells were numerous in the spiral colon of the Japanese field vole[14] and in the colon of the Korean hedgehog.[7] This difference

could be due to the gastric physiology between the species.

It is well known that somatostatin-immunoreactive cells show the widest distribution in the gastrointestinal tract. Somatostatin-immunoreactive cells were more numerous in the abdomen stomach especially in the pyloric region than in the intestine in the present study. These cells in the gastric mucosa were more numerous in the oxyntic glands than in the pyloric glands as reported in the cat, dog, man[1] and horse.[2] Ohara et al.[14] reported that these cells in the Japanese field vole were distributed in the whole gastrointestinal tract except for the pyloric stomach and that they were numerous in the fundic stomach, decreasing gradually to the rectum. However, these endocrine cells were most numerous in the pyloric gland of the sheep,[9] pig,[4] cat,[3] cow,[11] Korean native cattle[10] and goat.[12] Somatostatin-immunoreacive cells play important roles in gastric regulation: namely, the inhibition and stimulation of gastric acid secretion.[2] This difference between the species may be due to the difference of fixatives, regional difference and/or specificity of antiserum.

In the present study, Gas/CCK-immunoreactive cells were much more numerous in the pyloric region, also finding similarity in that of the rat, mouse, guinea pig, rabbit[15,16] and ruminants.[9~12]

In the present study, glucagon-immunoreactive cells were found in the whole gastrointestinal tract. Moderate numbers of these cells were found in the jejunum, ileum, colon and rectum, but few in the fundic region, the pyloric region and the duodenum. This regional distribution of these cells in the Korean native goat is generally similar to that of sheep.[9] However, the Korean native goat was different from the cat[3], horse[2] and Japanese field vole[14] in the presence of these cells in the only stomach, whereas the Korean native cattle,[10] cow[11] and goat[12] in the absence of these cells in the stomach. Larsson et al.[5] reported that glucagon-immunoreactive cells in the fundic stomach were rare as in man, rat and pig, but the peak of frequency was seen in the ileum of the rat, cat, dog and pig.

In the present study, PP-immunoreactive cells could be found in the ileum, colon and rectum. The regional distribution of PP-immunoreactive cells in the Korean native goat is generally similar to that of other mammals.[6,9~11,14] BPP-immunoreactive cells, however, could not be found in the whole gastrointestinal tract of the honey possum[17] but rarely in the small intestine of the caiman[18] and the echidna.[19] Whether PP-immunoreactive cells are absent in the stomach and small intestine of the Korean native goat, or whether the negative immunohistochemical staining is due to a difference in it's molecular form, is not yet clear.

Chromogranin-immunoreactive cells in the Korean native goat were distributed very numerously in the whole gastrointestinal tract especially in the fundic, pyloric and duodenal region. Recently, much attention has been given to the chromogranins[20], and a full sequence homology with several novel peptides was found within chromogranin A and B.[21,22] This clearly suggests a putative role for chromogranins as precursors of bioactive peptides. Chromogranin has also recently been shown to be present in all identifiable endocrine cell types in the adult human intestine.[23,24] In the present study, very numerous chromogranin-immunoreactive cells could be found in the whole gastrointestinal tract, and similar distributional pattern was seen in the Korean native cattle.[10]

This characteristic distribution and frequency of the immunoreactive cells in the gastrointestinal tract of the Korean native goat may reflect some of its herbivorous nature in digestion and gastric physiology. However, further morphological and physiological studies should be undertaken to make clear of the functional significance of the characteristic findings in the gastrointestinal tract of the Korean native goat.

Summary

The gastrointestinal endocrine cells of the Korean native goat were studied immunohistochemically, and 5-HT-, somatostatin-, Gas/CCK-, glucagon-, chromogranin- and PP- immunoreactive cells were revealed. The characteristic findings of the regional distribution and relative frequency of these immunoreactive cells in the gastrointestinal tract of the

Korean native goat were as follows.

5-HT-immunoreactive cells were more numerous in the small and large intestine than in the abdomen stomach. Somatostatin-immunoreactive cell were more numerous in the abdomen stomach than in the small and large intestine. Gas/CCK-immunoreactive cells were concentrated very numerously in the pyloric region with a few in the other regions. Moderate numbers of glucagon-immunoreactive cells were found in the small and large intestine, but a few of them were found in the abdomen stomach. Very numerous chromogranin- immunoreactive cells were detected throughout the gastrointestinal tract. PP-immunoreactive cells were observed moderate numbers in the large intestine with few in the ileum. No insulin-immunoreactive cell was found in the gastrointestinal tract.

Legends for figures

Figs 1a-d: (a, b) Somatostatin-, (c) 5-HT- and (d) glucagon-immunoreactive cells in the fundic region. a: ×120, b, c, d: ×500.

Figs 2a-c: (a) Somatostatin-, (b) 5-HT- and (c) glucagon-immunoreactive cells in the pyloric region. ×500.

Figs 3a,b: (a, b) Gas/CCK-immunoreactive cells in the pyloric region. a: ×120, b: ×500.

Figs 4a,b: (a, b) Chromogranin-immunoreactive cells in the pyloric region. a: ×120, b: ×500.

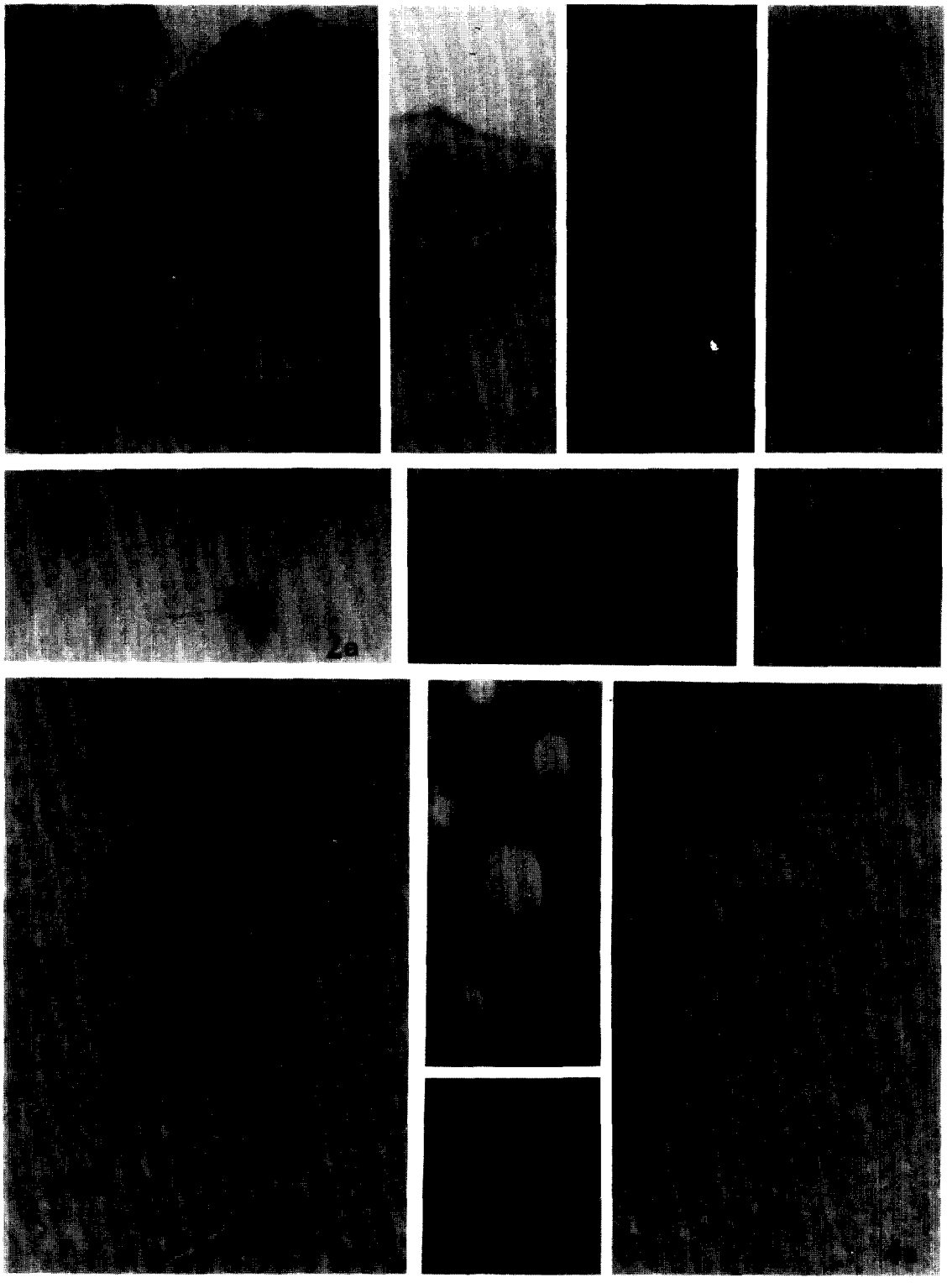
Figs 5a-e: (a) Somatostatin-, (b) 5-HT-, (c) Gas/CCK-, (d) glucagon-and (e) chromogranin-immunoreactive cells in the duodenum. ×500.

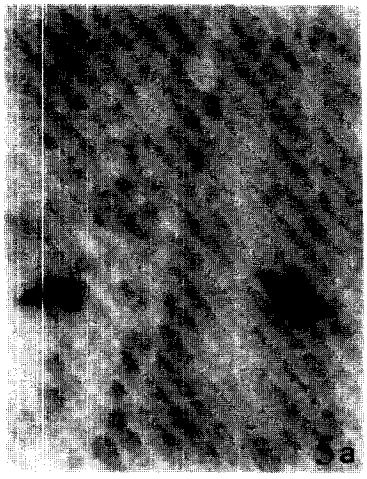
Figs 6a-e: (a) Somatostatin-, (b) 5-HT-, (c) Gas/CCK-, (d) glucagon-and (e) chromogranin-immunoreactive cells in the jejunum. ×500.

Figs 7a-f: (a) Somatostatin-, (b) 5-HT-, (c) Gas/CCK-, (d) chromogranin-, (e) PP-and (f) glucagon-immunoreactive cells in the ileum. ×500.

Figs 8a-g: (a) Somatostatin-, (b) 5-HT-, (c) glucagon-, (d) Gas/CCK-, (e) chromogranin-and (f, g) PP-immunoreactive cells in the colon. ×500 except the Fig b(×120).

Figs 9a-e: (a) Somatostatin-, (b) 5-HT-, (c) Gas/CCK-, (d) glucagon-and (e) chromogranin-immunoreactive cells in the rectum. ×500 except the Fig b(×120).

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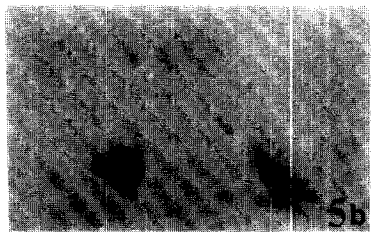
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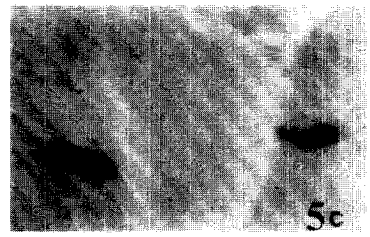
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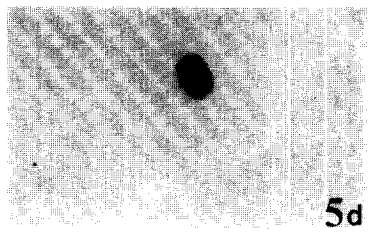
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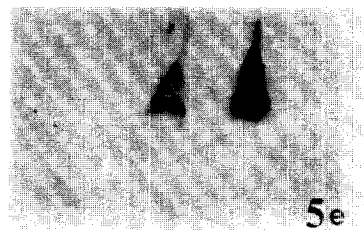
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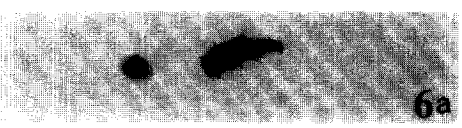
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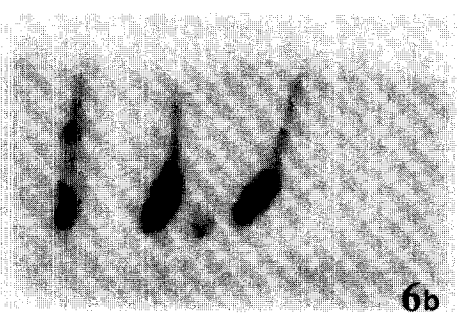
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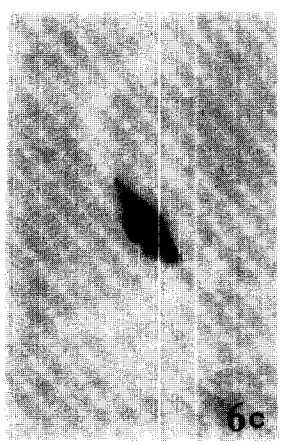
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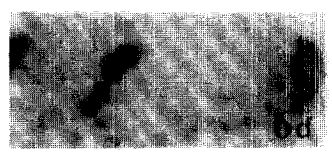
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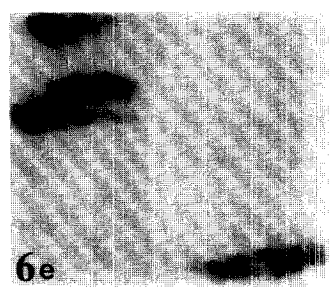
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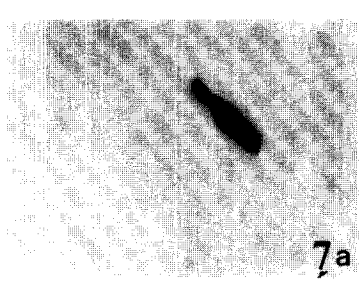
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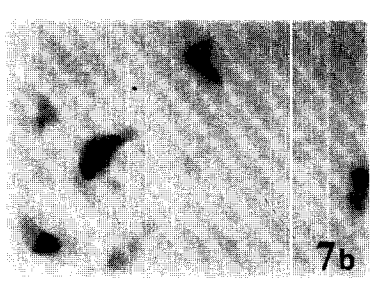
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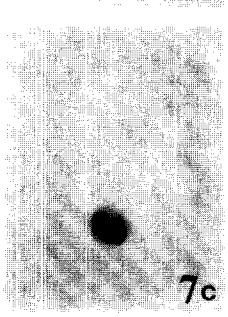
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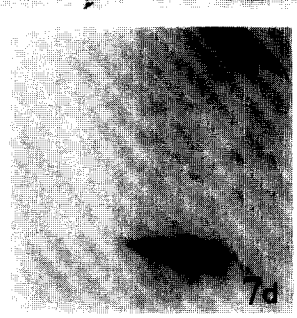
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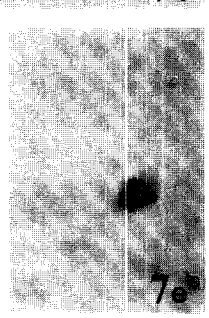
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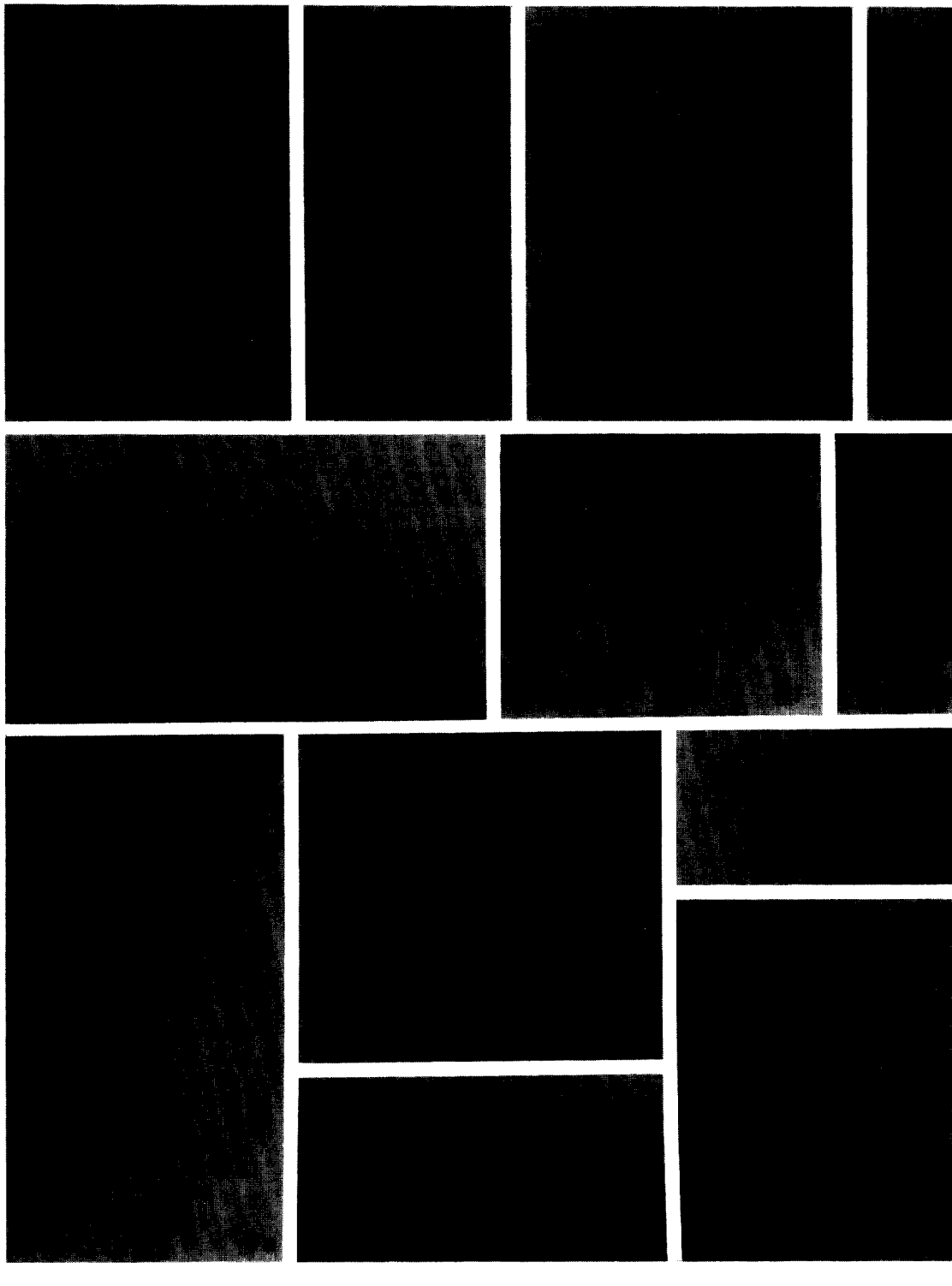

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