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博　士　論　文

Immunohistochemical study of enterochromaffin-like cell in human gastric mucosa.

（ヒト胃粘膜における ECL 細胞の免疫組織化学的検討）

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Abstract

Enterochromaffin-like (ECL) cell has been identified as the histamine-containing argyrophil cell in rat gastric mucosa and vigorously studied. However, there are few reports of the distribution of ECL cell in human stomach. The aim of this study was to reveal the precise distribution of ECL cell by immunohistochemical staining of histidine decarboxylase (HDC) and gastrin-cholecystokinin B receptor (CCK-BR) in human stomach and the correlation between their distribution and that of parietal cells. Thirty specimens of surgically resected stomach were used. Parietal cell, Grimelius-silver stain positive cell, gastrin, HDC and CCK-BR immunoreactive cell were studied by continuous cell counting in the restricted field along the lamina muscularis from the oral to the anal ends. The percentage of HDC immunoreactive cells of endocrine cells was smaller (15%) than that of previous report (35%) in the fundic region. HDC and CCK-BR immunoreactive cells were found not only in the fundic region, but also in the intermediate and pyloric regions. In the pyloric region, HDC and CCK-BR immunoreactive cells were found mainly in the mucosa with intestinal metaplasia. Double positive cells were also found, but only in small numbers. This suggests the ECL cell or its function would be present in the pyloric region.

Key words: enterochromaffin-like cell, histidine decarboxylase, gastrin-cholecystokinin B receptor, distribution, stomach

Introduction

Since Heidenhain observed chromaffin cell in 1870,[1] gastric endocrine cells such as enterochromaffin (EC), enterochromaffin-like (ECL), gastrin (G), somatostatin (D), D1, P, and X/A-like cell have been ultrastructurally and immunohistochemically characterized.[2-5] ECL cell was first reported as histamine-containing argyrophil cell in rat gastric mucosa[6-8] and abundant in the gastric oxyntic mucosa.[2,9,10] Its morphology and physiology were vigorously studied in rat.[11-14]

Although acetylcholine, gastrin, and histamine were identified as gastric acid secretagougues,[15,16] their role in mediating acid secretion is still controversial.[15-18]

It has been recognized that ECL cell has an important role in acid secretion, however, there are few reports regarding the precise distribution of ECL cell in the human gastric mucosa. Simonsson *et al.* reported that ECL cell represented 35% of the total endocrine cell in human oxyntic mucosa.[9] D'Adda *et al.* reported that the volume density of the ECL cell of the entire endocrine cell mass was 30% by electron microscopic analysis in 1989.[10] Rat ECL cell was reported to occupy 65% of total endocrine cell in the oxyntic mucosa.[2,9,10] This means that the ECL cell number in human is less than that in rat.

ECL cell was initially demonstrated by argyrophil-staining techniques. In recent years, ECL cell has been immunohistochemically identified using

antibodies against histamine, the histamine-forming enzyme, histidine decarboxylase (HDC), and vesicular monoamine transporter 2 (VMAT-2).[11-14,19] In addition, ECL cell has been known to possess the gastrin-cholecystokinin B receptor (CCK-BR).[20-22] Gastrin is well known to stimulate ECL cell and parietal cell through this CCK-BR to secrete histamine or HCl, respectively, but gastrin is also well known to act as a growth factor through this receptor.[23,24]

In the present study, the precise distribution of HDC and CCK-BR positive cells was studied immunohistochemically in the gastric mucosa and was compared with that of the parietal cell. The results showed that these HDC and CCK-BR positive cells were distributed even in the antral mucosa with inflammation.

Materials and methods

⟨Specimens⟩

Thirty specimens of surgically resected stomach from patients diagnosed with gastric cancer were examined (19 male and 11 female, aged between from 37 and 84 years, 13 cases of total gastrectomy and 17 cases of distal subtotal gastrectomy). All these specimens had tumor-free mucosa along the lesser curvature. Specimens were fixed in 10% buffered formalin and the slices along the lesser curvature were used for study and embedded in paraffin. Four micrometer thick sections were cut. Serial sections were placed on MAS-coated super-frost glass slides (Matsunami Glass Industries, Osaka, Japan). These sections were stained with hematoxylin and eosin (H.E.) and used for immunohistochemistry. Among these 30 cases, 21 cases had been used in a previous study.[25]

⟨Antibodies⟩

Rabbit polyclonal antibodies (Ab) against gastrin, HDC and CCK-BR (amino acids 206-219) were purchased from DAKO Japan (Kyoto, Japan), American Research products (Belmont, USA) and Acris antibodies (Hiddenhausen, Germany), respectively. No cross reaction between them except double positive cell preliminary confirmed. Then the positive cell of each immunostaining was specific.

〈Single immunostaining〉

Immunostaining for HDC and CCK-BR were performed with Biotin-free Tyramide Signal Amplification system (DAKO Japan, Kyoto, Japan).

Staining methods were as follows: 1) Incubation with 3% hydrogen peroxide to block endogenous peroxidase activity for 10 min. 2) Immerse in phosphate buffered saline (PBS). 3) Incubation in PBS with containing 1% bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA) to block nonspecific binding for 20 min. 4) Incubation with rabbit anti HDC Ab (1:800) for over night at 4℃ or rabbit anti CCK-BR Ab (1: 200) for 30 min at room temperature. 5) Washing 3 times in Tris-HCl buffered saline (pH7.6) containing Tween 20 (TBST). 6) Incubation with horse radish peroxidase (HRP) labeled anti-rabbit immunoglobulin goat antibody (1:100) for 15 min. 7) Washing 3 times in TBST. 8) Incubation with fluorescyl-tyramide and hydrogen peroxide (amplification regent) for 15min. 9) Washing 3 times in TBST. 10) Incubation with goat anti-fluorescein Ab conjugated with HRP for 15 min. 11) Washing 3 times in TBST. 12) Incubation with 3, 3' diaminobezidine (DAB) and counter-stain with hematoxylin.

Immunostaining for gastrin was performed according to the conventional method with using an LSAB2 kit (DAKO Japan).

As a control, negative staining was performed with PBS instead of the

primary antibody.

〈Double immunostaining〉

Double immunostaining for HDC and CCK-BR was performed as follows. After the first immunostaining for CCK-BR was visualized with DAB as described above, the sections were immersed in 10mM citrate buffer (pH6.0) and heated in a microwave oven at 500 W for 15 min to dissociate the first antibody. Then, the second immunostaining procedure for HDC was performed according to steps (2 – 8) aforedescribed. After step 8 (amplification regent), sections were incubated with alkaline phosphatase (AP)-conjugated anti-fluorescein sheep Fab fragments (1:1500, Roche, Penzberg, Germany) for 20 min. Then, AP activity was visualized with NBT /BCIP stock solution (1:200, Roche) for approximately 30 min at room temperature.

Double immunostaining for HDC and gastrin was performed in a similar manner to that aforedescribed. After the first immunostaining for HDC was visualized, and the first antibody dissociated, sections were incubated with anti gastrin antibody for 30 min. After washing with Tris-HCl buffered saline (pH 7.6), the slides were incubated with AP-conjugated goat anti-rabbit immunoglobulin (1:50, Dako) for 30 min. Then, AP activity was visualized with NBT /BCIP stock solution (1:200, Roche) for approximately 10 min at room temperature. Hematoxylin was used as counter-stain.

〈Grimelius-silver staining〉

All sections were stained by the Grimelius-silver method to demonstrate endocrine cells in the gastrointestinal tract.[26-28]

〈Cell counting〉

All sections were studied by light microscopy according our previous reports at x400 magnification with a 1-cm x 1-cm ocular micrometer (one square was defined as one field).[25,29] The distribution of parietal cell, Grimelius-silver stain positive cell, gastrin, HDC and CCK-BR immunoreactive cell in the stomach was studied by continuous cell counting in the restricted field along the lamina muscularis from the oral to anal ends. Gastric mucosa was divided into the fundic, intermediate and pyloric regions by H.E. staining as following. The region between the cardia or oral end and the point where the pyloric gland including pseudopyloric gland metaplasia was first recognized from the oral end was designated the fundic region. The region between the pyloric ring and the point where the fundic gland was first recognized from the anal end was designated the pyloric region. The region between these pyloric and fundic regions that had both pyloric gland including pseudopyloric gland metaplasia and fundic gland, and then was designated the intermediate region.

〈Appearance rate and Positive-field-average〉

In order to compare the distribution of parietal cells, HDC immunoreactive cells and CCK-BR immunoreactive cells in each case, two indices were used: Appearance rate (AR) and Positive-field-average

AR (%) = number of fields containing more than one positive cell x 100 / total number of fields containing no positive cell.

Positive-field-average = average cell count per field containing more than one positive cell.

〈Ratio of HDC immunoreactive cells to endocrine cells in the fundic region〉

In order to estimate the ratio of HDC immunoreactive cells to endocrine cells in the fundic region, the average cell count per field of HDC immunoreactive cells and Grimelius-silver stain positive cells in the fundic region was calculated, respectively.

〈Statistical analysis〉

Statistical analysis was carried out using Welch's *t* test and Mann-Whitney's U test. When the difference in mean values in each group was $P<0.05$, they were considered significant.

Results

〈Distribution of parietal cells〉

Thirty cases were classified into three types according to the AR and Positive-field-averages of parietal cells in all regions, rich type (cases 1-7, with a large number of parietal cells), mild type (cases 8-16, with a moderate number of parietal cells) and atrophic type (cases 17-30, with a small number of parietal cells) (Fig. 1). The average of the Positive-field-average in all regions was 30.93 ±4.30 (mean±SD) in the rich type, 12.64±3.77 in the mild type and 4.76±2.62 in the atrophic type. All the differences were significant ($P<0.01$).

The distribution of parietal cells is shown in Fig. 2. There were 0-115 parietal cells per field. There were a high number of parietal cells in the fundic region, and a few parietal cells in the pyloric region. The total number of the counted field of each case was within the range from 288 to 733.

〈Histological findings〉

These 30 cases were classified into three types as mentioned above. Among the cases of the rich type, the fundic gland in the fundic region was rather preserved. Among the cases of the mild type, the mucosal atrophy with inflammation became evident with the decrease of the parietal cell in the fundic region. Among the cases of the atrophic type, fundic region was only recognized in a few cases and showed severe inflammation and the marked decrease of the

parietal cell. Concerning to the mucosal inflammation, most of cases showed the mucosal inflammation and the intestinal metaplasia, but they differed case by case and site by site, from negative to severe in all regions. In the pyloric region, the mucosal atrophy with inflammation and the decrease of the pyloric gland was recognized in all cases, although the degree of them differed case by case and site by site, as well. In the intermediate region, the mucosal atrophy with inflammation was recognized in the same degree as that of the pyloric region and the pyloric gland including pseudopyloric gland metaplasia often intermingled with the fundic gland. These findings revealed the mucosal atrophy with inflammation and the intestinal and/or pseudopyloric gland metaplasia was spread through most regions except the fundic region of the rich type, and had no correlation with the classification described here, except the fundic region.

〈Distribution of gastrin immunoreactive cells〉

The distribution of gastrin immunoreactive cells is shown in Fig. 3. There were 0-74 gastrin immunoreactive cells per field. Most of gastrin immunoreactive cells were located in the pyloric and intermediate regions. However, there were a few gastrin immunoreactive cells in the fundic region. In the case 1, gastrin immunoreactive cells were recognized around the neck area between the pit and base. These cells were found not only in the pyloric and intermediate regions, but also in a part of fundic region.

〈Distribution of HDC immunoreactive cells〉

There were 0-53 HDC immunoreactive cells per field (Fig. 4). HDC immunoreactive cells were found not only in the fundic region, but also in the intermediate and pyloric regions. In the fundic region, HDC immunoreactive cells were predominantly located in the lower half of the oxyntic mucosa (Fig. 5). In the pyloric region, HDC immunoreactive cells were found in the mucosa with intestinal metaplasia and pyloric gland including pseudopyloric gland metaplasia. There were few gastrin-HDC double positive cells (Fig. 6).

The average cell count per field was 2.13±1.11 in the fundic region and 3.07 ±2.00 in the pyloric region. The number of HDC immunoreactive cells in the fundic region was smaller than that in the pyloric region. The average cell count per field in the fundic region in the rich type was the lowest (1.66±0.89).

In the pyloric region, the average AR in the rich type was 72.23±16.80 which was significantly higher than that in the atrophic type, 43.65±16.53 (P<0.01). The average of the Positive-field-average of the rich type was 4.21± 1.66 and of the atrophic type was 5.11±1.87. The Positive-field-average was relatively higher in the atrophic type than in the rich type, but the difference was not significant.

〈Distribution of CCK-BR immunoreactive cells〉

There were 0-83 CCK-BR immunoreactive cells per field (Fig. 7). In the

fundic region of the rich type, the distribution of CCK-BR immunoreactive cells was similar to that of parietal cells. Although CCK-BR immunoreactivity was found mainly in parietal cells in the fundic region (Fig. 8a), in other regions it was mostly found in the mucosa with intestinal metaplasia and pyloric gland including pseudopyloric gland metaplasia (Fig. 8b).

In the pyloric region, the average AR of CCK-BR immunoreactive cells was 19.19±14.64 and was significantly more frequent than that of parietal cells, 9.04 ± 16.60 ($P<0.05$) and the average of the Positive-field-average of CCK-BR immunoreactive cells was 3.06±1.25 and was significantly higher than that of parietal cells, 1.93±1.90 ($P<0.01$).

〈Distribution of HDC and CCK-BR double positive cells〉

There were a small number of double positive cells, 0-9 per field. Double positive cells were found not only in the fundic region, but also in the intermediate and pyloric regions (Figs. 9, 10).

〈Ratio of HDC immunoreactive cells to endocrine cells in the fundic region〉

The average cell count per field of HDC immunoreactive cells in the fundic region of the rich type was 0.60-2.82 (1.66±0.89). The average cell count per field of Grimelius-silver stain positive cells in the fundic region of the rich type was 6.00-18.56 (11.05±4.33). The average percentage of HDC immunoreactive

cells to Grimelius-silver stain positive cells in the fundic region of the rich type was approximately 15%.

Discussion

In recent years, ECL cell has been identified specifically by immunohistochemistry. In the present study, the authors tried to estimate the ECL cell distribution using 3 indices, average cell count per field, average AR and average of the Positive-field-average. These 3 indices were very useful to compare the each immunohistochemical results.

First, HDC was reported as an ECL cell marker.[2,11] Although the distribution of HDC immunoreactive cells in the lower half of the oxyntic mucosa was similar to the previous reports,[2,9] the percentage of HDC immunoreactive cells of endocrine cells was low, approximately 15 % of the endocrine cell mass in the present study. HDC was reported to have a short half-life and to be rapidly adjusted to histamine production in rat.[30,31] Approximately 80% reduction of HDC was also reported in mice after 24 hours of fasting and HDC immunostaining was difficult to demonstrate in ECL cell of fasted animals.[32] Since the gastric specimens used in this study were collected from the surgically resected stomach, it is possible that the fasting conditions prior to the surgical intervention might affect the HDC immunostainability. In addition, ECL cell frequency was previously reported to be 35% by subtracting the number of the EC cell from the number of Sevier-Munger positive cell.[9] The different calculating method applied here could be a cause for this discrepancy. Although it was difficult to confirm the accurate number of HDC immunoreactive cells in the

human stomach, the present study confirmed the wide distribution of HDC immunoreactive cells in the human stomach even after the fasting condition.

In the present study, there were large numbers of HDC immunoreactive cells in the pyloric region. Hunyady *et al.* suggested that G cell synthesized and secreted gastrin and histamine in the rat stomach.[33] On the other hand, Zhao *et al.* reported that some anti HDC antibodies reacted immunohistochemically with G cell in the rat stomach among five anti HDC antibodies which they used, but some did not.[34] In our study, there were few double positive cells for gastrin and HDC. Gastrin or HDC immunoreactive cells were recognized individually in the mucosa with intestinal metaplasia and/or pyloric gland including pseudopyloric gland metaplasia.

The degree of mucosal inflammation differed from mild to severe and did not correlate with the classification used in the present study. The mucosal inflammation was commonly recognized in the pyloric region. Among most of the mild and atrophic type, the mucosa showed intestinal metaplasia and pseudopyloric gland metaplasia in the pyloric and intermediate regions. This meant all of the studied stomachs showed mucosal atrophy, mild or severe, in the pyloric and intermediate regions. Mutoh *et al.* reported that ECL cell density increased markedly in the oxyntic mucosa of Cdx-2 transgenic mice with intestinal metaplasia. ECL cells were mainly located in the basal portion of the intestinal metaplasia, although the number of ECL cells in the oxyntic mucosa of

wild type mice was small.[35] The present results suggested that the HDC immunoreactive cell number also increased in the mucosa with inflammation and the intestinal and pseudopyloric gland metaplasia in human.

The CCK-BR was reported to be expressed on ECL cell and parietal cell.[20,21,36-39] Although CCK-BR immunoreactive cells were found in all regions in the present study, CCK-BR immunoreactive cells were similarly distributed to parietal cells in the fundic region of the rich type. Then most of CCK-BR immunoreactive cells would be parietal cells in the fundic region. Schmitz F. *et al.* reported that CCK-BR mRNA was expressed in neuroendocrine cells in human antral mucosa by *in situ* RT-PCR.[40] In contrast, Kulaksiz H *et al.* showed that no expression of CCK-BR was found in the gastric antral mucosa by immunohistochemistry and *in situ* RT-PCR of biopsy specimens.[38] These results suggest that CCK-BR immunohistochemistry is difficult. In the present study, the authors confirmed CCK-BR immunostain was faintly positive by conventional method and tried to enhance its sensitivity by ascertaining the most suitable combination of staining procedures to avoid the cross reactivity as mentioned in the Materials and methods. The enhancement by the tyramide signal amplification was applied and successful in the present study. The similar distribution of CCK-BR immunoreactive cells to that of parietal cells in the fundic region of the rich type reveals the reliability of the CCK-BR immunostaining in the present study and means that the positive immunostaining of CCK-BR in the

present study would be compatible with the report of Schmitz F. *et al.*[40] The AR and Positive-field-average of CCK-BR were higher than that of parietal cells in the pyloric region. These results suggested that CCK-BR immunoreactive cells in the pyloric region involved other cell types than parietal cells. Gastrin was well known to play a role as a growth factor in epithelial cell proliferation.[23, 24] CCK-BR positive cells in the pyloric region were found in the mucosa with intestinal metaplasia and pyloric gland including pseudopyloric gland metaplasia in the present study and then these CCK-BR positive cells might play a role in the mucosal regeneration.

Double positive cells were also recognized in the pyloric region. This result suggested that ECL cell or its function would be present in the pyloric region. To confirm ECL cell in the pyloric region, further studies are needed using other techniques such as *in situ* hybridization.

In conclusion, the present study showed that the percentage of HDC immunoreactive cells to endocrine cells in human surgically resected stomach was lower (15%) than that of previous reports (35%). Both HDC and CCK-BR immunoreactive cells were found even in the pyloric region. These positive cells were found mainly in the mucosa with intestinal metaplasia and pyloric gland including pseudopyloric gland metaplasia in the pyloric region. The distribution of these immunoreactive cells suggests that ECL cell or its function would be present in the pyloric region and different from that of rodent.

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References

1 Heidenhain R. Untersuchungen über den Bau der Labdrüsen. *Arch Mikr Anat* 1870; **6**: 368-406.

2 Sundler F, Häkanson R. Gastric endocrine cell typing at the light microscopic level. In: Häkanson R, Sundler F, eds. *The stomach as an endocrine organ.* Amsterdam: Elsevier, 1991; 9-26.

3 Öberg K. Gastric neuroendocrine cells and secretory products. *Yale J Biol Med* 1998; **71**: 149-154.

4 Bordi C, D'adda T, Azzoni C, Ferraro G. Classification of gastric endocrine cells at the light and electron microscopical levels. *Microsc Res Tech* 2000; **48** :258-271.

5 Solcia E, Rindi G, Buffa R, Fiocca R, Cappella C. Gastric endocrine cells: types, function and growth. *Regul Pept* 2000; **93**: 31-35.

6 Häkanson R, Lilja B, Owman CH. Properties of a new system of amine-storing cells in the gastric mucosa of the rat. *Eur J Pharmacol* 1967; **1**: 188-199.

7 Häkanson R, Owman CH. Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffine-like cells of gastric mucosa. *Life Sci* 1967; **6**: 759-766.

8 Häkanson R, Owman CH. Argyrophilic reaction of histamine-containing epithelial cells in murine gastric mucosa. *Experientia* 1969; **25**: 625-626.

9 Simonsson M, Eriksson S, Häkanson R *et al.* Endocrine cells in the human oxyntic mucosa. A histochemical study. *Scand J Gastroenterol* 1988; **23**: 1089-1099.

10 D'adda T, Bertelé A, Pilato FP, Bordi C. Quantitative electron microscopy of endocrine cells in oxyntic mucosa of normal human stomach. *Cell Tissue Res* 1989; **255**: 41-48.

11 Kubota H, Taguchi Y, Tohyama M *et al.* Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa. *Gastroenterol* 1984; **87**: 496-502.

12 Häkanson R, Chen D, Sundler F. The ECL cells. In: Jonson LR, ed. *Physiology of the gastrointestinal tract, 3rd edn.* New York: Reven Press, 1994; 1171-1184.

13 Zhao CM, Jacobsson G, Chen D, Häkanson R, Meister B. Exocytotic proteins in enterochromaffin-like (ECL) cells of the rat stomach. *Cell Tissue Res* 1997; **290**: 539-551.

14 Zhao CM, Chen D, Lintunen M, Panula P, Häkanson R. Secretory organelles in ECL cells of the rat stomach: an immunohistochemical and electron-microscopic study. *Cell Tissue Res* 1999; **298**: 457-470.

15 Valle JD, Todisco A. Gastric secretion. In: Yamada T, ed. *Textbook of gastroenterology, 3rd edn.* Philadelphia: Lippincottwilliams & Wilkins, 1999; 278-319.

16 Lindström E, Chen D, Norlén P, Andersson K, Häkanson R. Control of gastric acid secretion: the gastrin-ECL cell-parietal cell axis. *Comp Biochem Physiol A* 2001; **128**: 505-514.

17 Code CF. Histamine and gastric secretion. In: Wolatenholme GEW, O'Conner CJ, eds. *Ciba foundation symposium on histamine.* London: J&A, 1956: 189-219.

18 Grossman MI, Konturek SJ. Inhibition of acid secretion in dog by metiamide, a histamine antagonist acting on H_2 receptors. *Gastroenterol* 1974; **66**: 517-521.

19 Rindi G, Paolotti D, Fiocca R, Wiedenmann B, Henry JP, Solcia E. Vesicular monoamine transporter 2 as a marker of gastric enterochromaffin-like cell tumors. *Virchows Arch* 2000; **436**: 217-223.

20 Roche S, Gusdinar T, Bali JP, Magous R. "Gastrin" and "CCK" receptors on histamine- and somatostatin-containing cells from rabbit fundic mucosa-II. *Biochem Pharmacol* 1991; **42**: 771-776.

21 Wank SA. Cholecystokinin receptors. *Am J Physiol* 1995; **269**: G628-G646.

22 Ding XQ, Lindström E, Häkanson R. Cholecyctokinin-B/gastrin receptor blockade suppresses the activity of rat stomach ECL cells. *Phamacol Toxicol* 1997; **81**: 19-25.

23 Nakajima T, Konda Y, Izumi Y *et al.* Gastrin stimulates the growth of gastric pit cell precursors by inducing its own receptors. *Am J Physiol* 2002; **282**: G359-G366.

24 Dockray G, Dimaline R, Varro A. Gastrin: old hormone, new function. *Eur J Physiol* 2005; **449**: 344-355.

25 Tanaka-Shintani M, Watanabe M. Distribution of ghrelin-immunoreactive cells in human gastric mucosa: comparison with that of parietal cells. *J Gastroenterol* 2005; **40**: 345-349.

26 Grimelius L. A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc Med Upsal* 1968; **73**: 243-270.

27 Grimelius L. Silver stains demonstrating neuroendocrine cells. *Biotech Hstochem* 2004; **79**: 37-44.

28 Cetin Y. Chromogranin A immunoreactivity and Grimelius' argyrophilia. a correlative study in mammalin endocrine cells. *Anat Embryol* 1992; **185**: 207-215.

29 Kijima I, Nakagawa M, Shintani M, Watanabe M. The distribution of endocrine cells in the surgically resected stomach. *J Gastroenterol* 2002; **37**: 396-397.

30 Dartsch C, Chen D, Häkanson R, Persson L. Histidine decarboxylase in rat stomach ECL cells: relationship between enzyme activity and different molecular forms. *Regul Pept* 1999; **81**: 41-48.

31 Tanaka S. Physiological function mediated by histamine synthesis. *Yakugaku Zasshi* 2003; **123**: 547-559. (In Japanese)

32 Torbergsen K, Wiksén H, Johansen K, Rahimipoor S, Falkmer UG, Zhao CM. Immunoreactivity of gastric ECL and A-like cells in fasted and fed rats and mice. *Biotech Histochem* 2005; **80**: 21-30.

33 Hunyady B, Zölyomi A, Hoffman BJ, Mezey É. Gastrin-producing endocrine cells: A novel source of histamine in the rat stomach. *Endocrinol* 1998; **139**:4404-4415

34 Zhao CM, Chen D, Dornonville de la Cour C, Lindqvist A, Persson L, Häkanson R. Histamine and histidine decarboxylase are hallmark features of ECL cells but not G cells in rat stomach. *Regul Pept* 2004; **118**: 61-66

35 Mutoh H, Hakamata Y, Sato K *et al.* Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem Biophys Res Commun* 2002; **294**: 470-479.

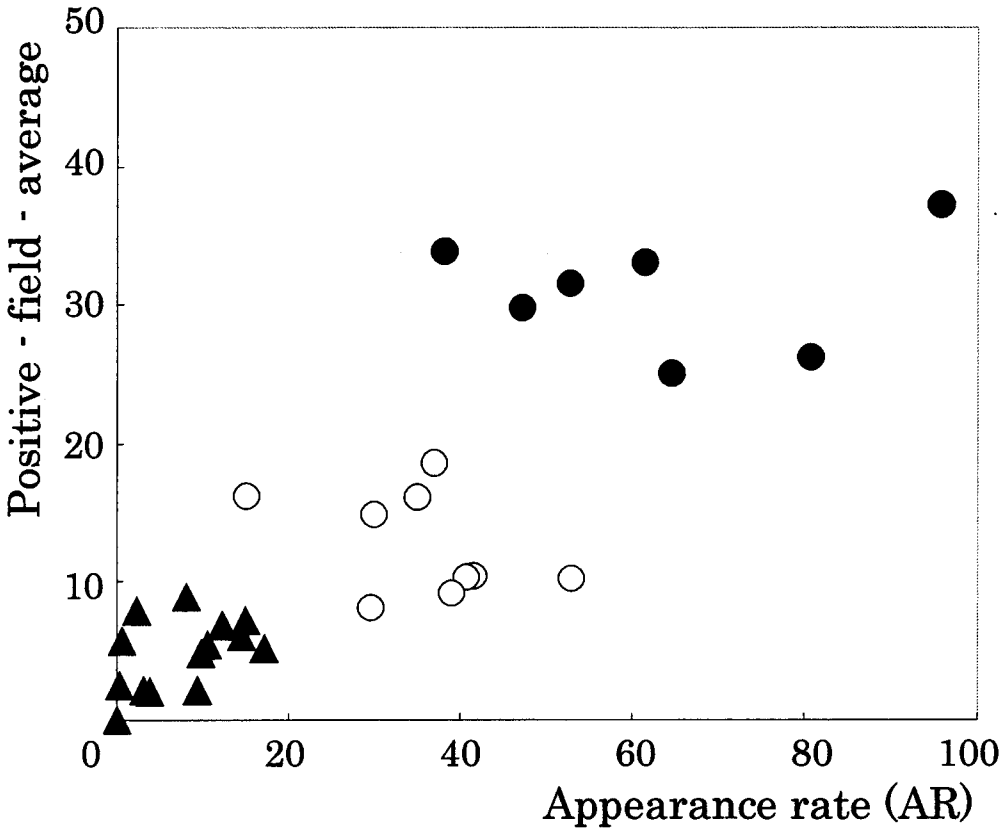
36 Chiba T, Fisher SK, Agranoff BW, Yamada T. Autoregulation of muscarinic and gastrin receptors on gastric parietal cells. *Am J Physiol* 1989; **256**: G356-G363.

37 Tarasova NI, Romanov VI, Da Silva PP, Michejda CJ. Numerous cell targets for gastrin in the guinea pig stomach revealed by gastrin/CCK_B receptor localization. *Cell Tissue Res* 1996; **283**: 1-6.

38 Kulaksiz H, Arnold R, Göke B *et al.* Expression and cell-specific localization of the cholecystokinin B/ gastrin receptor in the human stomach. *Cell Tissue Res* 2000; **299**: 289-298.

39 Hur K, Kwak K, Lee HJ *et al.* Expression of gastrin and its receptor in human gastric cancer tissues. *J Cancer Res Clin Oncol* 2006; **132**: 85-91.

40 Schmitz F, Göke MN, Otte JM *et al.* Cellular expression of CCK-A and CCK-B/ gastrin receptor in human gastric mucosa. *Regul Pept* 2001; **102**: 101-110.



●, Rich type ; ○, Mild type ; ▲, Atrophic type.

Figure 1. Relationship between Appearance rate and Positive-field-average of parietal cells. All differences were significant ($P<0.01$).

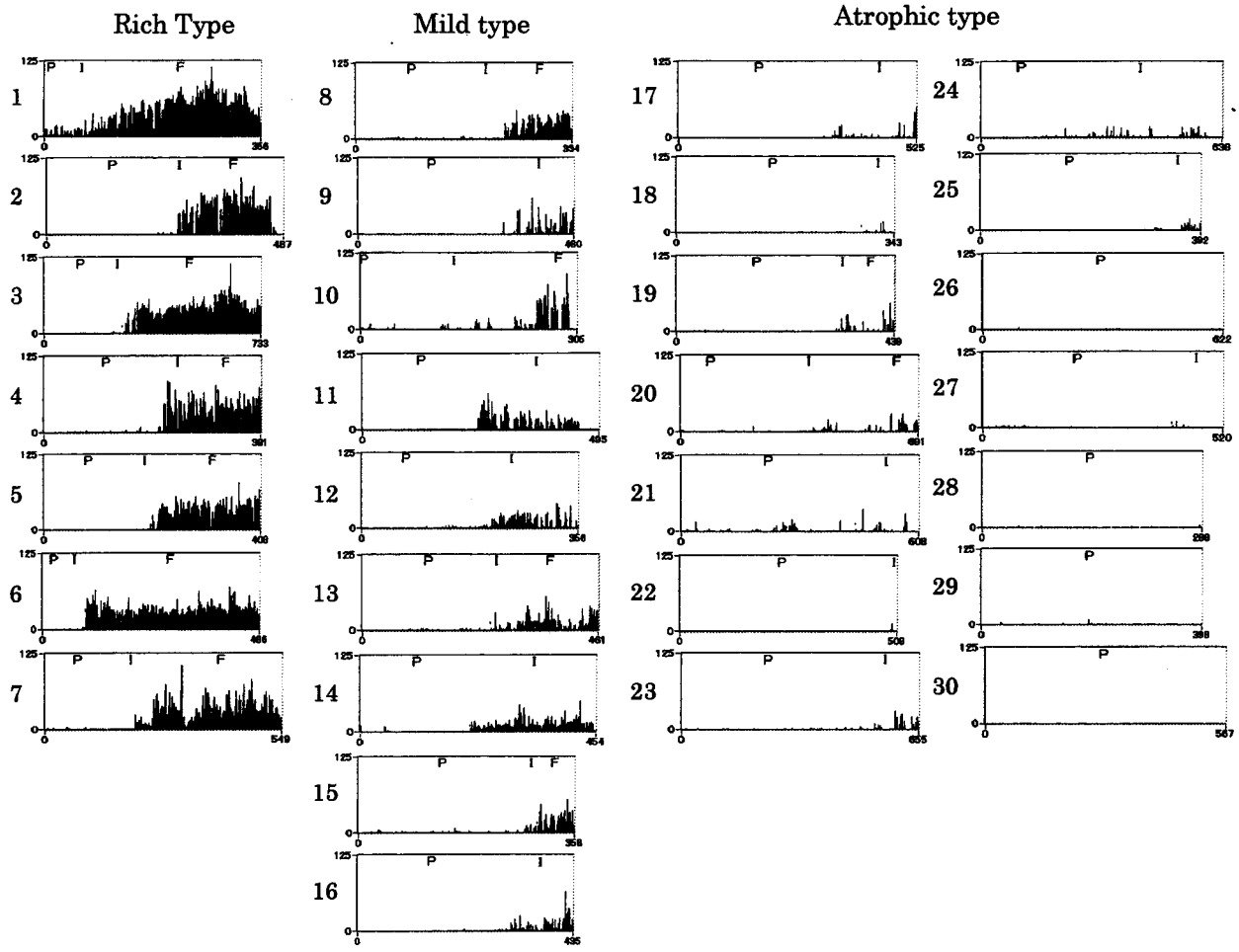


Figure 2. Distribution of parietal cells. Vertical axes: positive cell counts per field. Horizontal axes: number of counted fields along the slice of the lesser curvature. The total number of counted fields for each stomach is shown at the end on the right. P, pyloric region; I, intermediate region; F, fundic region. Long horizontal axes, total gastrectomy; short horizontal axes, subtotal gastrectomy.

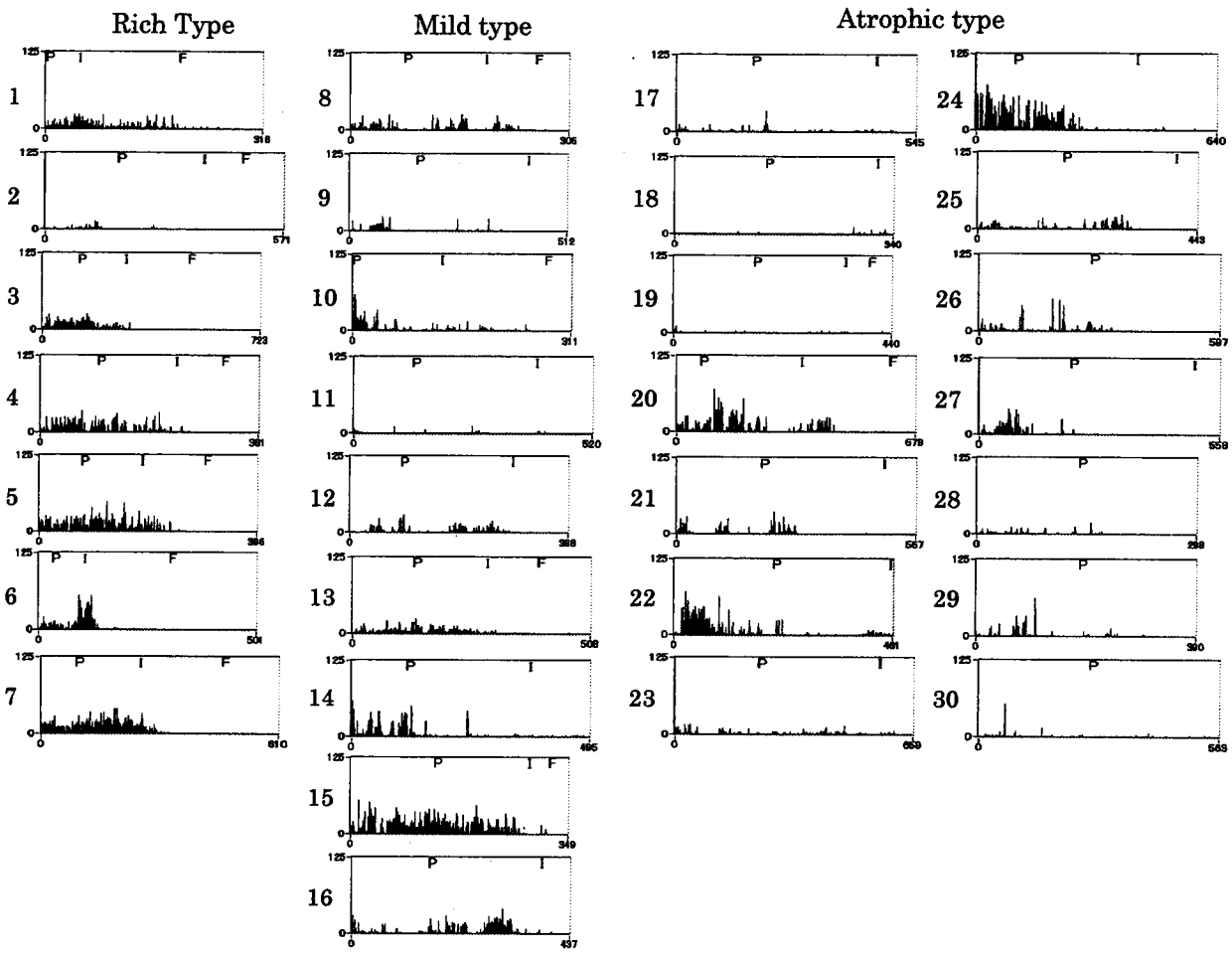


Figure 3. Distribution of gastrin immunoreactive cells. Vertical axes: positive cell counts per field. Horizontal axes: number of counted fields along the slice of the lesser curvature. The total number of counted fields for each stomach is shown at the end on the right. P, pyloric region; I, intermediate region; F, fundic region. Long horizontal axes, total gastrectomy; short horizontal axes, subtotal gastrectomy. Most of gastrin immunoreactive cells were located in the pyloric and intermediate regions.

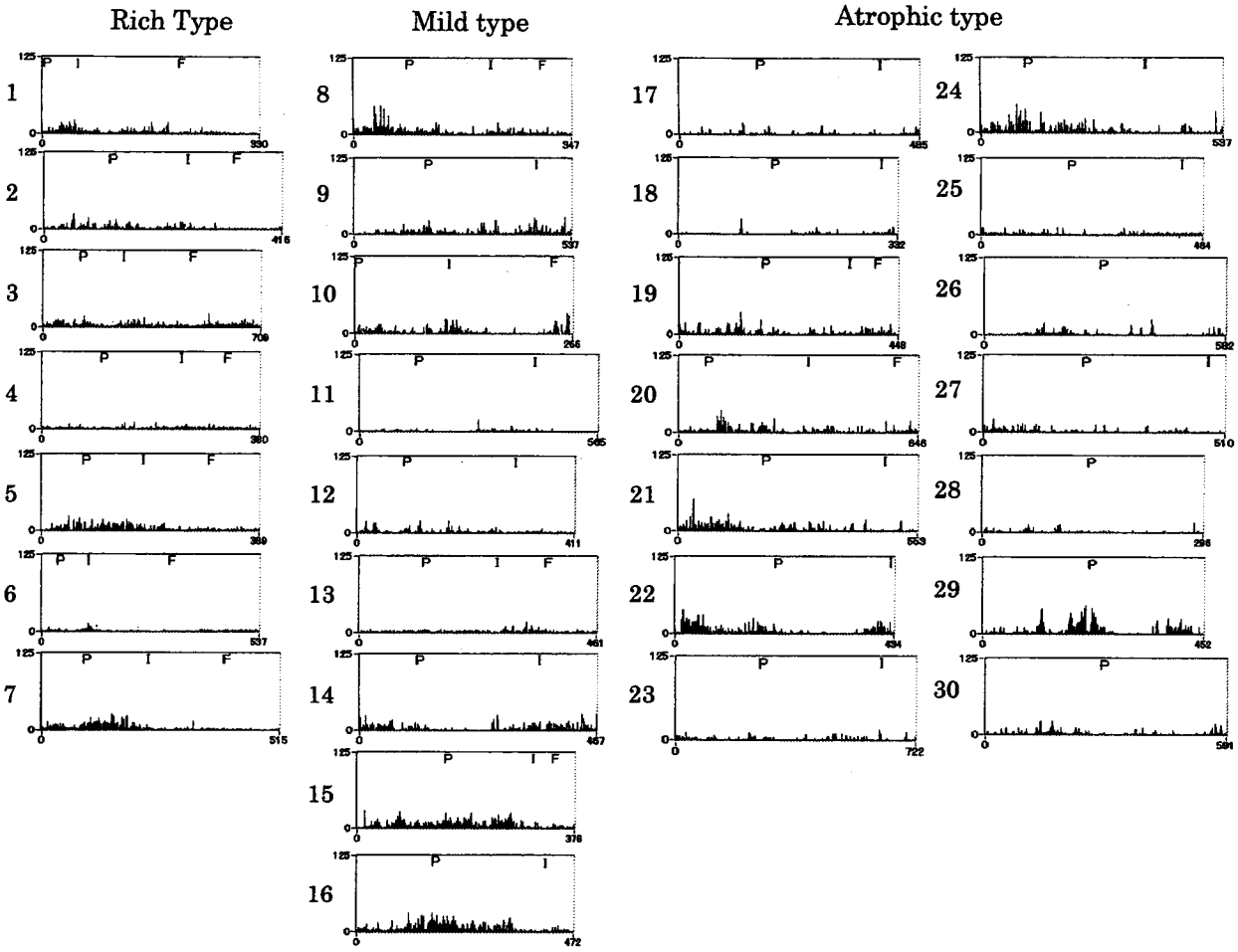


Figure 4. Distribution of Histidine decarboxylase (HDC) immunoreactive cells. Vertical axes: positive cell counts per field. Horizontal axes: number of counted fields along the slice of the lesser curvature. The total number of counted fields for each stomach is shown at the end on the right. P, pyloric region; I, intermediate region; F, fundic region. Long horizontal axes, total gastrectomy; short horizontal axes, subtotal gastrectomy. The number of HDC immunoreactive cells in the fundic region was lower than that of the pyloric region.

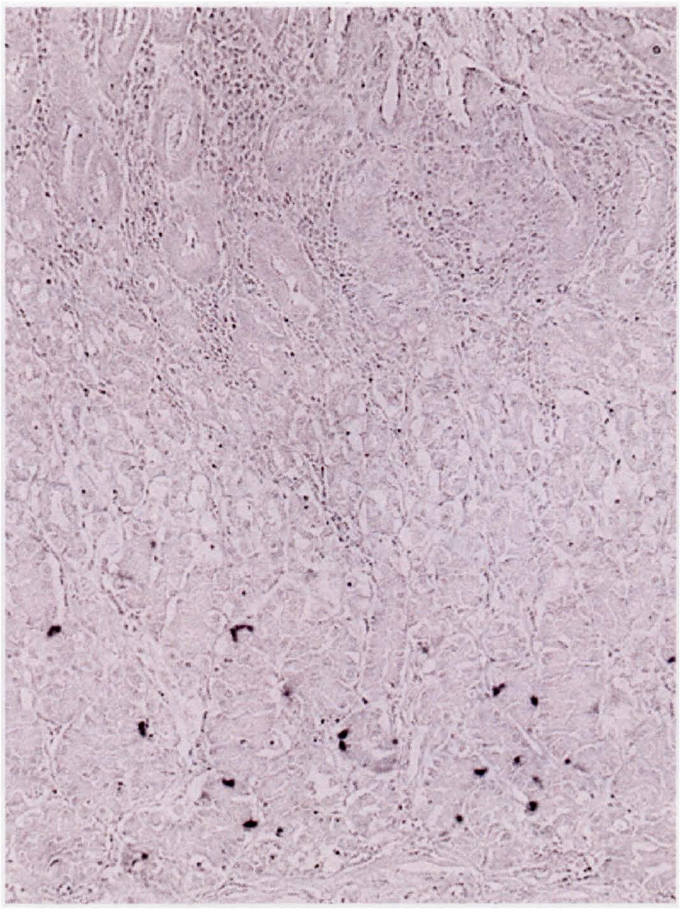


Figure 5. Histidine decarboxylase immunoreactive cells in the fundic region. Positive cells were located predominantly in the lower half of the oxyntic mucosa. Counter-stain with hematoxylin. Low-power field.

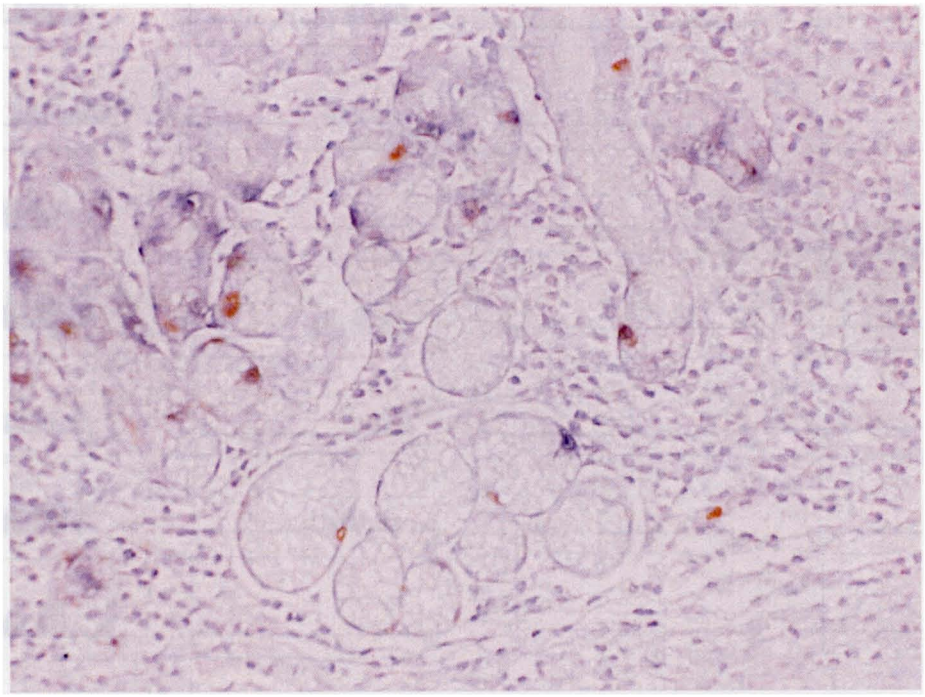


Figure 6. Double immunostaining of Histidine decarboxylase (HDC) (brown) and gastrin (blue). HDC immunoreactive cells were located in the mucosa with intestinal metaplasia and the pyloric gland. There were few gastrin-HDC double positive cells. Counter-stain with hematoxylin. Low-power field.

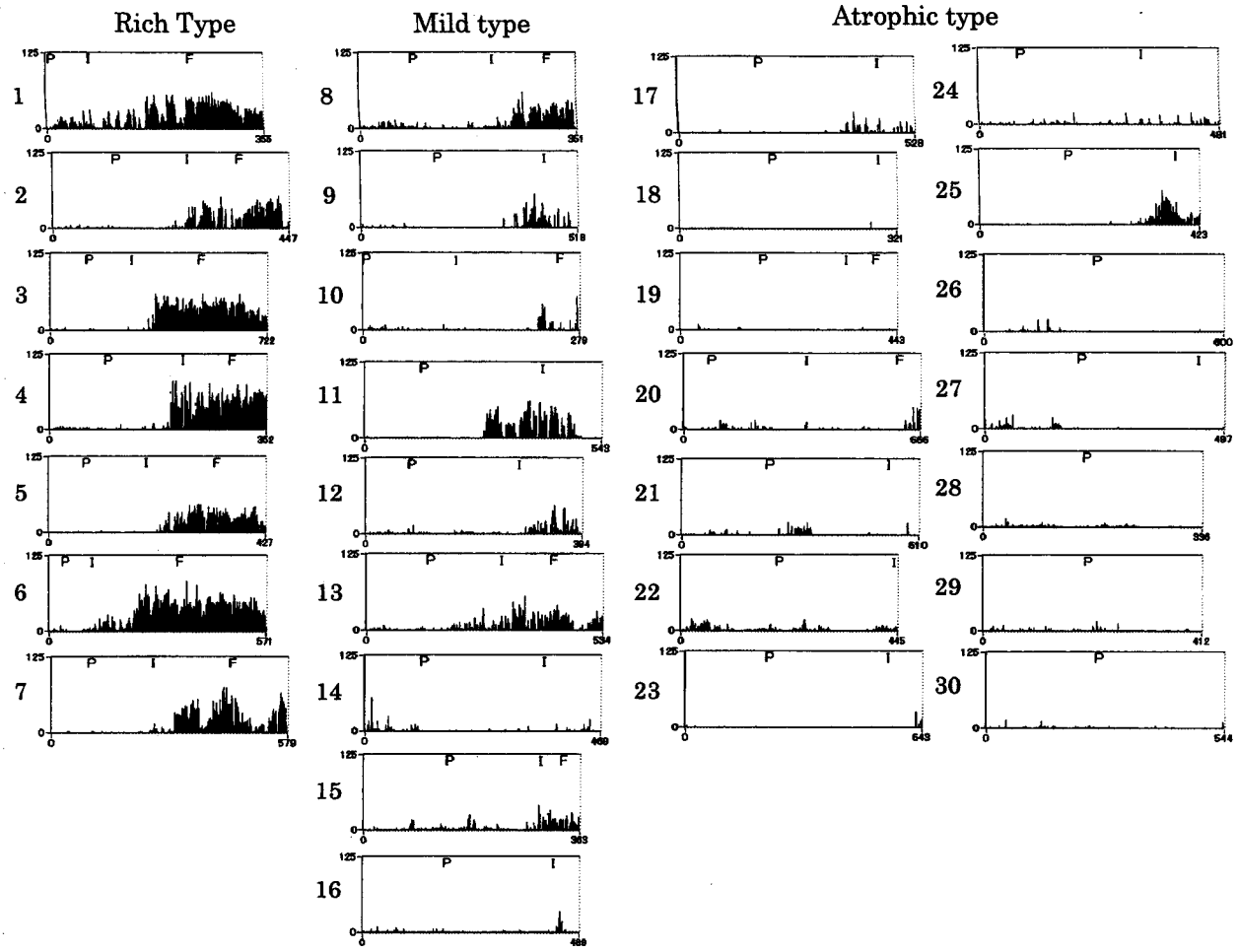


Figure 7. Distribution of gastrin-cholecystokinin B receptor (CCK-BR) immunoreactive cells. Vertical axes: positive cell counts per field. Horizontal axes: number of counted fields along the slice of the lesser curvature. The total number of counted fields for each stomach is shown at the end on the right. P, pyloric region; I, intermediate region; F, fundic region. Long horizontal axes, total gastrectomy; short horizontal axes, subtotal gastrectomy. In the fundic region of the rich type, the distribution of CCK-BR immunoreactive cells was similar to that of the parietal cells. CCK-BR immunoreactive cells were found in all regions.

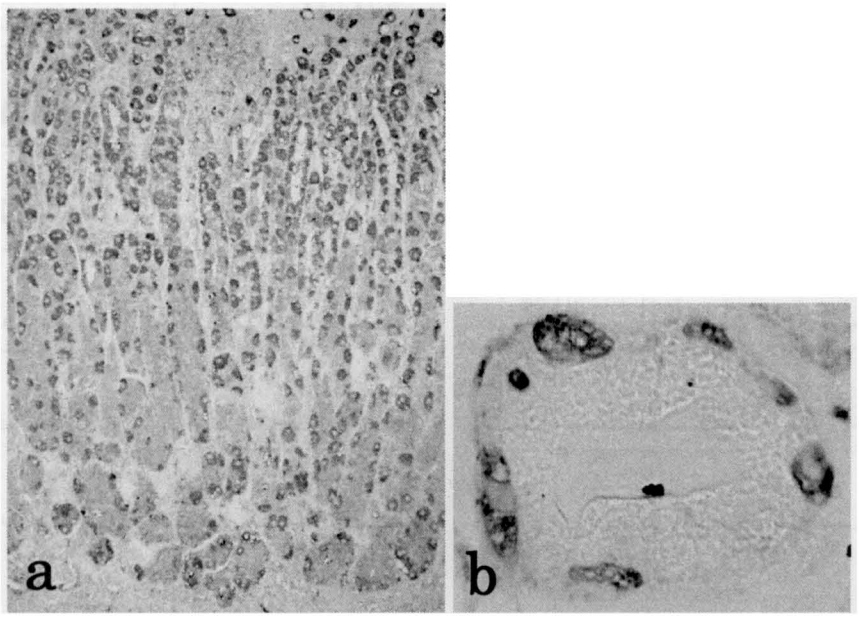


Figure 8. Gastrin-cholecystokinin B receptor immunoreactive cells. Counter-stain not done. (a) Positive cells were mainly found in parietal cells in the fundic region. Low-power field. (b) Positive cells were found in the pseudopyloric gland in the pyloric region. High-power field.

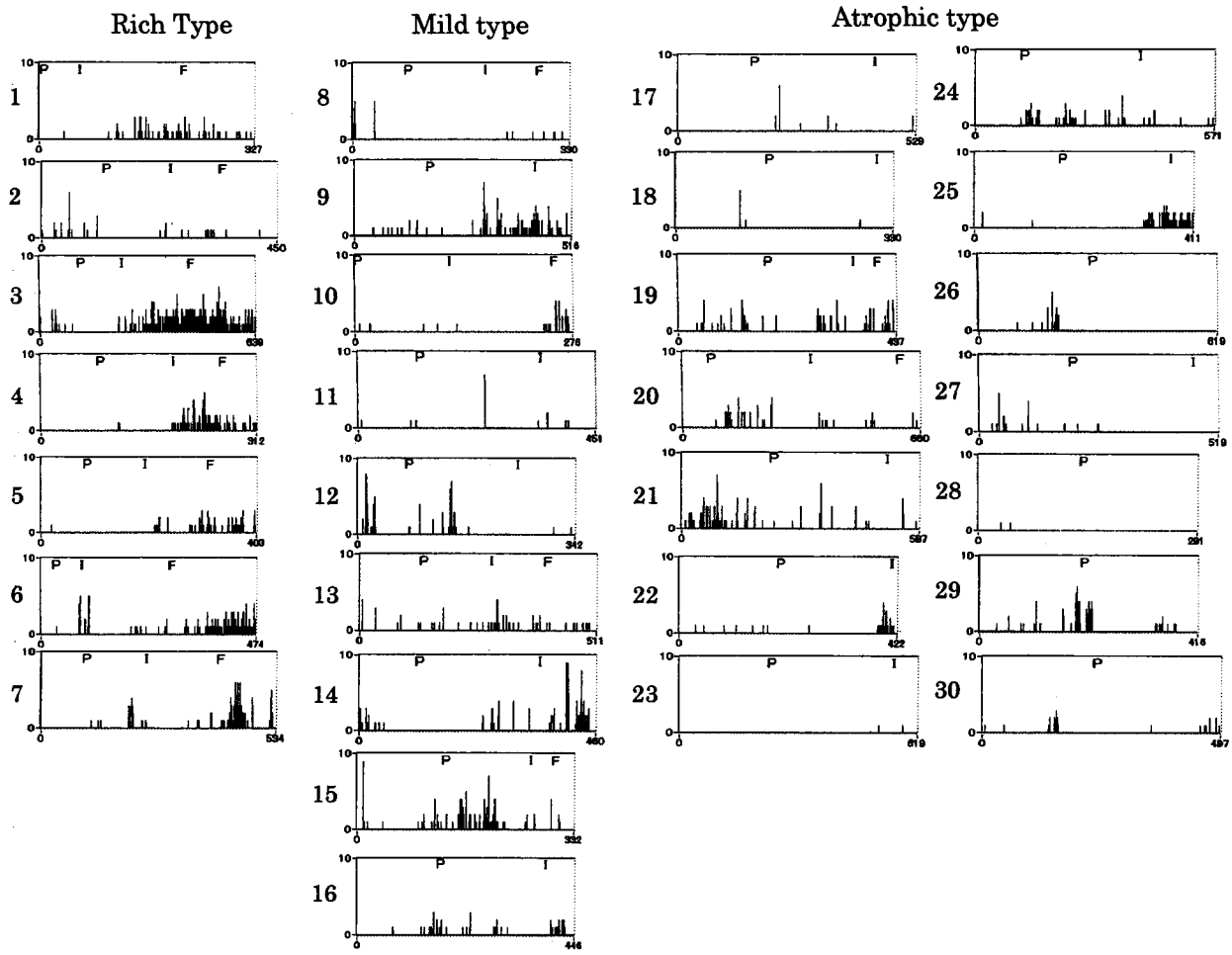


Figure 9. Distribution of histidine decarboxylase and gastrin·cholecystokinin B receptor double positive cells. Vertical axes: positive cell counts per field. Maximal value is 10 and different from other Figures 2,3,4,7. Horizontal axes: number of counted fields along the slice of the lesser curvature. The total number of counted fields for each stomach is shown at the end on the right. P, pyloric region; I, intermediate region; F, fundic region. Long horizontal axes, total gastrectomy; short horizontal axes, subtotal gastrectomy. Double positive cells were recognized in a small number and distributed in all regions.

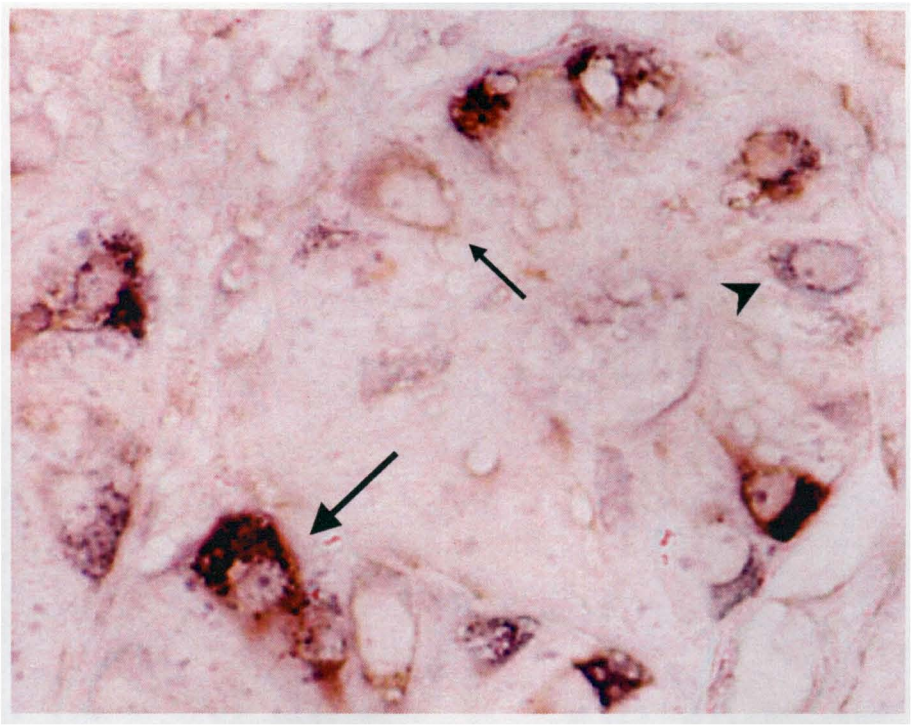


Figure 10. Double immunostaining of histidine decarboxylase (HDC) (arrow head) and gastrin-cholecystokinin B receptor (CCK-BR) (small arrow). HDC and CCK-BR double positive cells (large arrow) were also found in the pseudopyloric gland in the pyloric region. High-power field. Counter-stain not done.