Acta Med. Nagasaki 39 : 30－39

Distribution and Localization of Endocrine Cells in the Human Gastro-intestinal Tract －In Relation to Histogenesis of Rectal Carcinoid－

Kouki MUTA★, Minoru ITSUNO★, Kazuya MAKIYAMA★, Toru NAKAGOE★★, Masuho HARAGUCHI★★★, and Kenichiro INOUE★★★★

★ Second Department of Internal Medicine, Nagasaki University School of Medicine
★★ First Department of Surgery, Nagasaki University School of Medicine
★★★ Sasebo Municipal Hospital
★★★★ Inoue Hospital

The distribution of endocrine cells in the human intestine was studied by immunostaining using the labeled avidin-biotin technique. The total number of endocrine cells was abundant in the proximal portion of the small intestine and the distal portion of the large intestine. Gastrin, cholecystokinin, and motilin immunoreactive cells were localized in the proximal portion of the small intestine. Peptide YY, serotonin, and glucagon/glicentin immunoreactive cells were distributed more abundantly in the lower large intestine. The serotonin and somatostatin concentrations in the colonic mucosa determined by high-performance liquid chromatography and radioimmunoassay were higher in the distal than proximal portion of the large intestine, being in correlation with the distribution of serotonin and somatostatin immunoreactive cells estimated by immunostaining. Therefore, the regional differences in the number of endocrine cells and the mucosal concentrations of the hormones probably reflect differences in the physiological functions of different regions of the gut.

Not many endocrine cells with unknown peptides and animes and immature endocrine cells were present in the lower large intestine. Therefore, the frequent occurrence of carcinoids in the rectum is difficult to explain by the quantitative dominance of endocrine cells alone in the rectal mucosa, and other factors are considered to need evaluation.

Key Words : Endocrine cell, Gastro-intestinal tract, Rectal carcinoid.

Introduction

Endocrine cells are distributed throughout the digestive tract[1),2)]. Although different regions of the digestive tract have been found to have different types and numbers of endocrine cells[3)], it has been difficult to estimate the distribution and localization of endocrine cells present in different regions. Recently, Chromogranin A (CGA), a large acidic protein originally discovered in the adrenal medulla, was found in endocrine cells throughout the gastro-entero-pancreatic system[4),5),6)]. This protein has been reported to be a convenient specific marker for endocrine cells[7)]. To determine the distribution and localization of endocrine cells in each region of the gut, we examined mucosal endocrine cells of the human small and large intestine immunohistochemically using antibodies against CGA. We also examined them for the number and type of immunoreactive cells using eight antibodies. The concentrations of somatostatin and serotonin in the normal mucosa of the large intestine were measured by radioimmunoassay and high-performance liquid chromatography (HPLC), respectively, and compared with the immunohistochemical results to determine their correlation.

We also examined immunohistochemically 17 cases of rectal carcinoids, which occur at a relatively high frequency among the gastrointestinal carcinoids. Furthermore we examined the number and type of endocrine cells in the rectum, duodenum and ileum where gastrointestinal carcinoids occur most frequently next to the stomach.

Materials and Methods

Samples of normal tissues were obtained at abdominal operations from the small and large intestine. The samples were judged to be normal by conventional histopathological examinations. The date on the patients who provided the samples are shown in Table 1. The samples of each portion were collected from 4 to 27 patients. Seventeen cases of rectal carcinoids were obtained by endoscopic polypectomy. The normal and carcinoid samples were fixed in 10 % neutral buffered formalin, embedded in paraffin, cut into 6 μm-thick sections, and subjected to immunohistochemistry.

Table 1. Descriptions of the Patients for Immunostaining

Gut segment examined	No.of patients	Age (yr)	
		Median	Range
Duodenum	21(14M 7F)	71	43-85
Jejunum	5(3M 2F)	59	52-67
Ileum	6(2M 4F)	58	29-80
Distal ileum	24(16M 8F)	69	36-84
Ascending colon	22(17M 5F)	73	59-84
Transverse colon	27(15M 12F)	65	36-83
Descending colon	15(10M 5F)	61	39-77
Sigmoid colon	27(18M 9F)	62	31-83
Rectum	25(19M 6F)	64	40-84

Table 2. Details of Antisera

Antisera	Dilution	Source
Cholecystokinin	1:1000	Cambridge Research Biochemicals
Gastrin	1:1000	DAKO
Glucagon/Glicentin	1:4000	MILAB
Motilin	1:1000	ICN Immuno Biologicals
Pancreatic polypeptide	1:1000	DAKO
Peptide YY	1:1000	Peninsula Laboratories,INC
Somatostatin	1:1000	DAKO
Serotonin	1:1000	DAKO
Chromogranin A	1:1000	DAKO

Table 3. Descriptions of the Patients for the Tissue Concentration of Somatostatin and Serotonin

Gut segment examined	No. of patients	Age (yr)	
		Median	Range
Ascending colon	9(2M 7F)	70	62-88
Transverse colon	4(1M 3F)	57	30-72
Sigmoid colon	12(8M 4F)	66	43-83
Rectum	7(3M 4F)	58	48-78

The details of the antisera used are shown in Table 2. After deparaffinization, the section were immunostained by the labeled avidin-biotin (LAB) technique. Each section was incubated in a drop of the primary antiserum at an adequate concentration at 4 ℃ for 24 hours. The secondary antiserum (biotinylated swine anti-rabbit immunoglobulins or biotinylated goat anti-mouse immunoglobulins) and peroxidase-conjugated streptavidin were reacted at 1 : 500 for 60 minutes each. The peroxidase was visualized using DAB/H_2O_2 (0.7mM diaminobenzidine-HCL in 0.002% H_2O_2). Controls were incubated with a non-immune serum of the same animal species at the same dilution instead of the primary antiserum, or without the primary and secondary antisera.

The numbers of endocrine cells were counted using a microscope at a X 200 magnification equipped with a lattice sectioned to 0.25 mm-squares (divisions). Cells within 20 to 30 divisions were counted for each section and the means calculated. The numbers of endocrine cells were expressed as the numbers per 1 cm of the digestive tract. Only endocrine cells located in the glands perpendicular to the mucosa with a clearly visible nucleus were counted.

The results of the immunostaining of rectal carcinoid were classified as follows. (1) -: no positive cells, (2) (+): $<1\%$ positive cells, (3) +: 1-5% positive cells, (4) ++: 5-50% positive cells, and (5) +++: $>$ 50% positive cells.

The concentrations of somatostatin and serotonin were examined in the normal mucosa from the ascending, transverse, and sigmoid colon and the rectum obtained at abdominal operations. The findings obtained on the patients who provided the samples are shown in Table 3. The mucosa was isolated from the samples by microdissection and its weight was measured. For somatostatin measurement, the mucosal samples were extracted in a five times excess of 0.5 N acetic acid, homogenized at 0 ℃, incubated in boiling water for 3 minutes followed by immediate cooling in ice, centrifuged at 15,000 rpm at 4℃ for 5 minutes, and subjected to radioimmunoassay. For serotonin measurement, the mucosal samples were extracted in 0.2 N $HClO_4$ containing 0.1 % (w/v) Na_2EDTA, homogenized at 0 ℃, centrifuged at 15,000 rpm at 4 ℃ for 5 minutes, and subjected to HPLC.

The results are expressed as the mean ± standard error of mean unless otherwise indicated. Statistical tests were carried out using the repeated variance analysis method. For statistically significant differences, the combinations with significant differences were identified by the least significance difference method.

Results

Endocrine cells in the mucosa are found mainly in the lower part of the gland. They are flask-shaped and secretory granules are located on the basal side of the cell (Figures 1a, 1b, 1c). The presence of secretory granules in these endocrine cells was also confirmed by immunoelectron microscopy by the post-embedding method using protein A gold (Figures 2a, 2b, 2c).

The cells immunoreactive to CGA, a marker of the endocrine cell, were abundant in a large area from the small intestine to the large intestine, especially in the proximal portion of the small intestine and the distal portion of the large intestine (Figure 3). Cholecystokinin (CCK) immunoreactive cells were found only in the duodenum (Figure 4). The cells immunoreactive to gastrin and motilin were observed only in the duodenum and jejunum, but not in the other portions of the small and large intestine (Figure 5, Figure 6). The cells immuno-

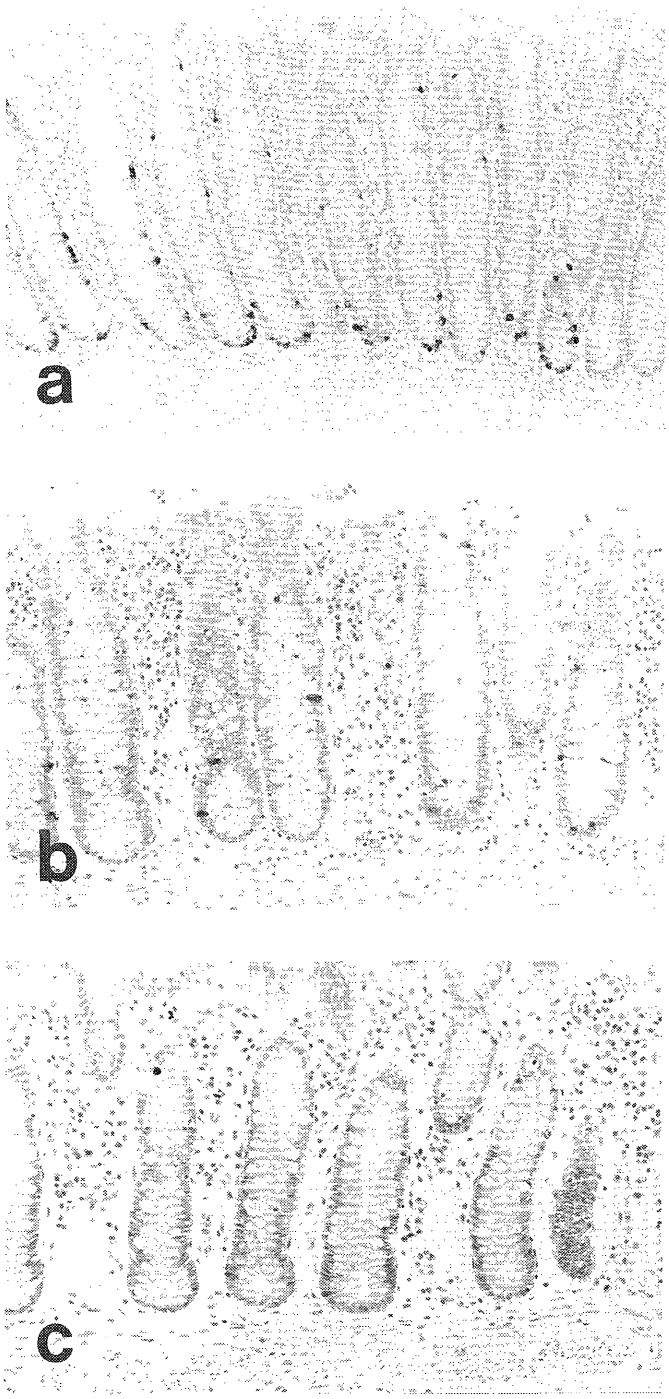

Figure 1a-c. Normal rectal mucosa immunostained for chromogranin A (a), peptide YY (b) and somatostatin (c). The immunoreactive cells are found predominantly in the lower part of the glands. (LAB technique, a = x 160, b = x 160, c = x 160)

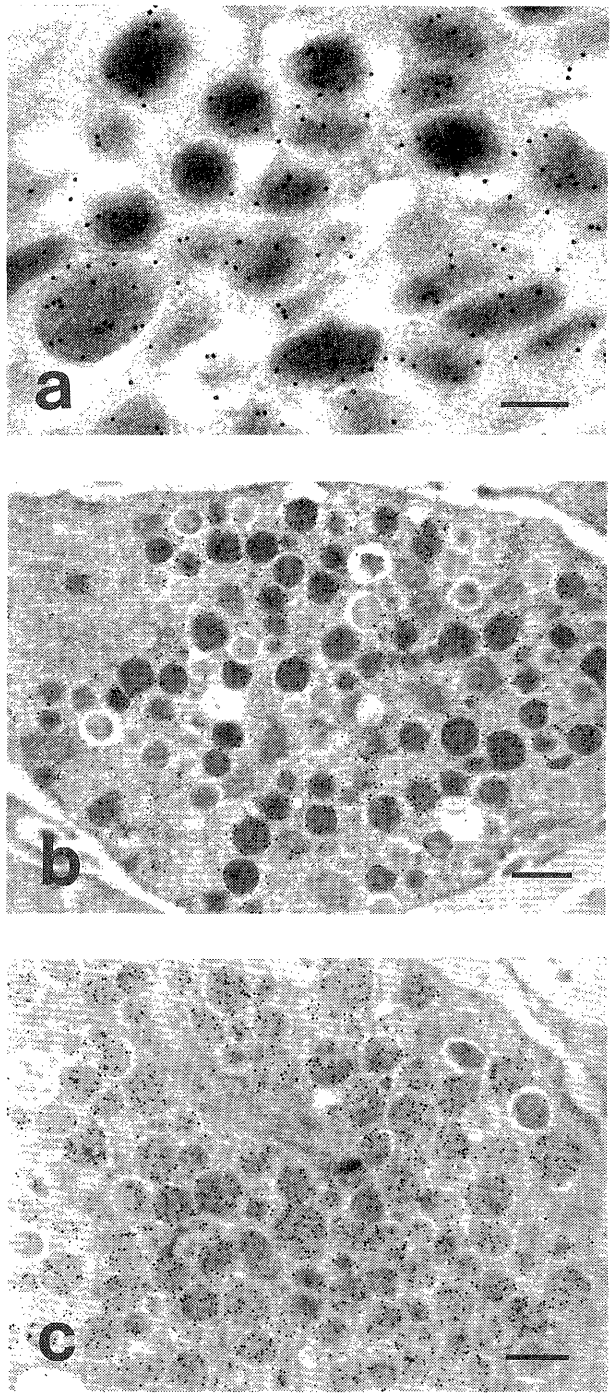

Figure 2a-c. Electron micrographs of secretory granules of endocrine cells in the human rectum intensely labelled with immunogold particles after incubation with antichromogranin A serum (a), antipeptide YY serm (b) and antisomatostatin serum (c). (Protein A-gold technique, a = x 40,000 b = x 15,000 c = x 15,000) a : Bar = 200nm, b and c : Bar = 500nm.

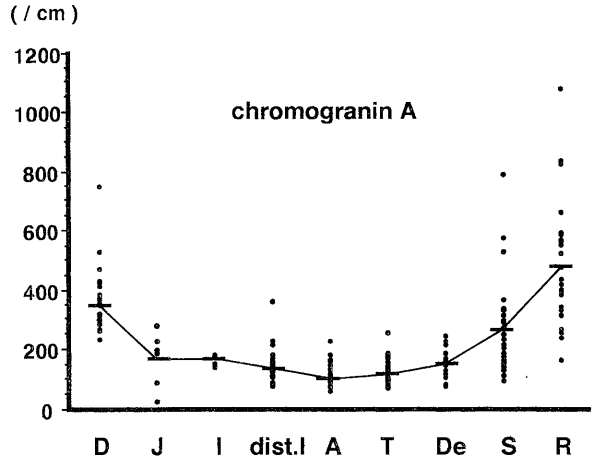

Figure 3. Number and distribution of chromogranin A immunoreactive cells in various regions of the intestines. Abbreviations: D = duodenum, J = jejunum, I = ileum, dist I = distal ileum, A = ascending colon, T = transverse colon, De = descending colon, S = sigmoid colon, R = rectum. -: mean

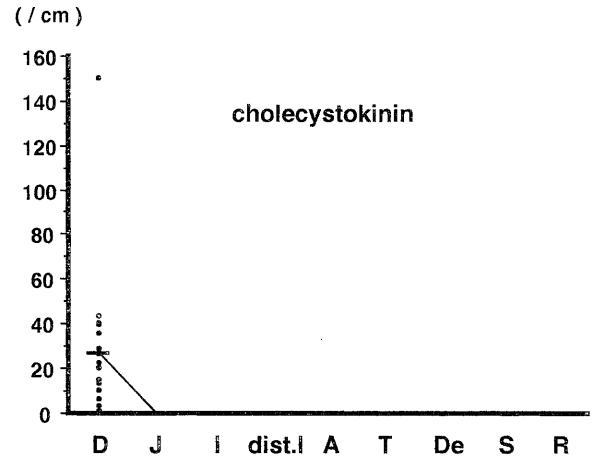

Figure 4. Number and distribution of cholecystokinin immunoreactive cells.

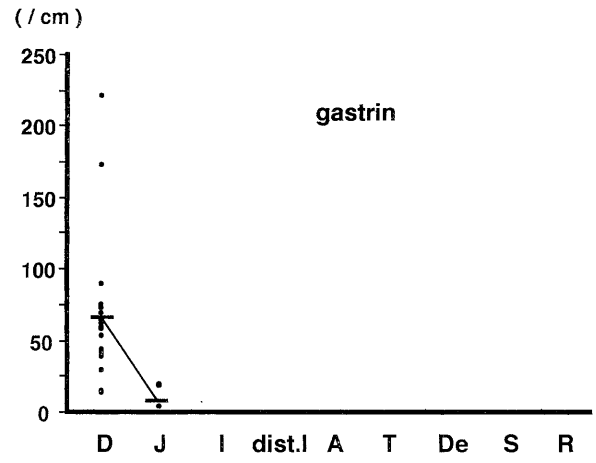

Figure 5. Number and distribution of gastrin immunoreactive cells.

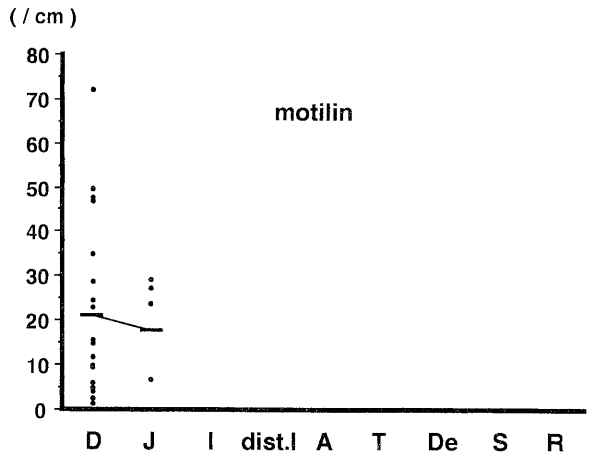

Figure 6. Number and distribution of motilin immunoreactive cells.

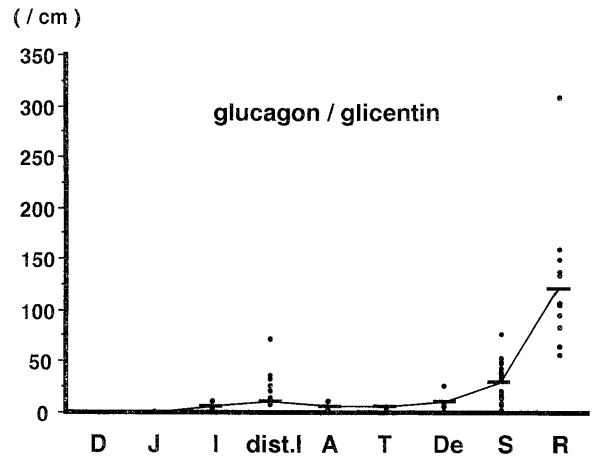

Figure 7. Number and distribution of glucagon/glicentin immunoreactive cells.

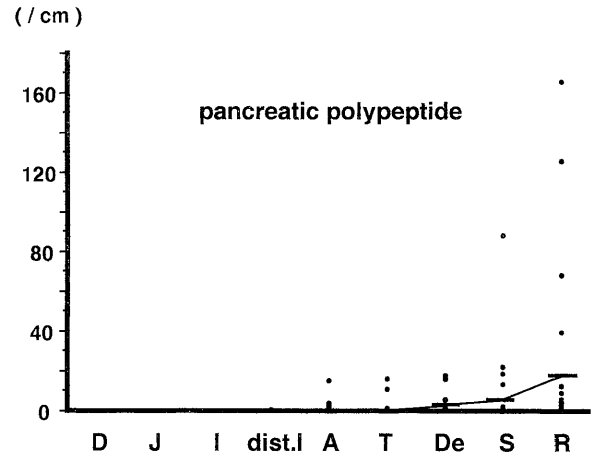

Figure 8. Number and distribution of pancreatic polypeptide immunoreactive cells.

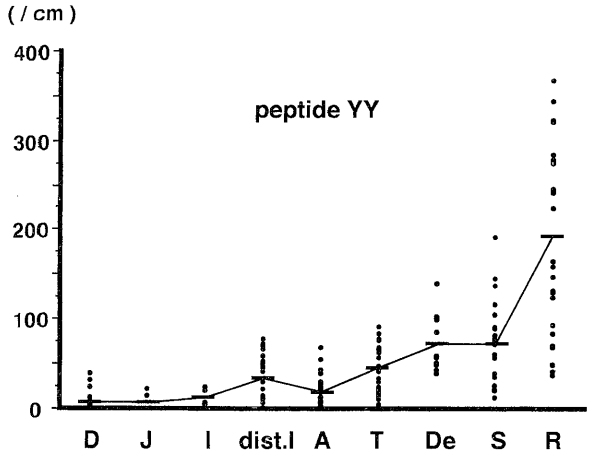

Figure 9. Number and distribution of peptide YY immunoreactive cells.

reactive to glucagon/glicentin were distributed from the jejunum to the large intestine, but they were few in number and tended to be more abundant in the distal portion of the large intestine than in the other regions (Figure 7). A small number of pancreatic polypeptide (PP) immunoreactive cells were found only in the large intestine being more abundant in its distal portion (Figure 8).

Peptide YY (PYY), serotonin and somatostatin immunoreactive cells were distributed throughout the small and large intestine. PYY immunoreactive cells were frequently observed in the region from the distal portion of the ileum to the large intestine, particularly in the distal portion of the large intestine (Figure 9). Serotonin immunoreactive cells were frequently observed in the duodenum, distal portion of the ileum and the large intestine, and were particularly abundant in the distal portion of the large intestine (Figure 10). Somatostatin

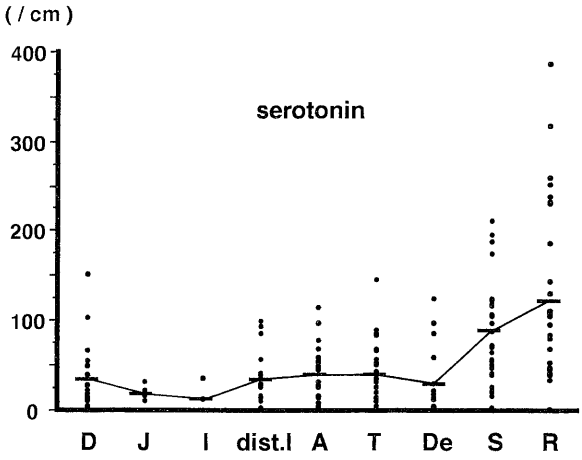

Figure 10. Number and distribution of serotonin immunoreactive cells.

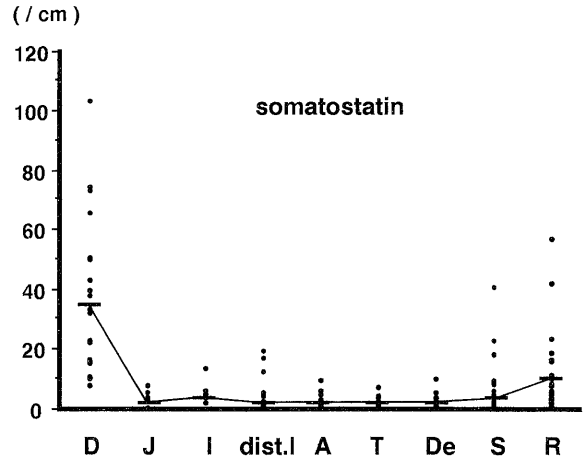

Figure 11. Number and distribution of somatostatin immunoreactive cells.

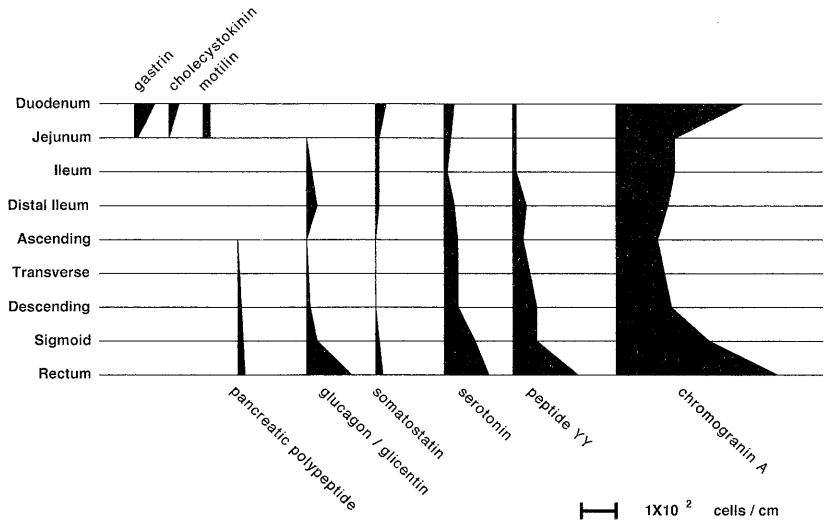

Figure 12. The regional distribution and frequency of the different endocrine cell types and chromogranin A immunoreactive cells.

immunoreactive cells existed primarily in the duodenum and distal portion of the large intestine (Figure 11). Figure 12 summarizes the distributions in the gut of the cells immunoreactive to CGA, and eight kinds of peptides and serotonin. The number of endocrine cells as expressed by the CGA immunoreactive cells was large in the proximal portion of the small intestine and the distal portion of the large intestine. Of the cells immunoreactive to the eight peptides and serotonin, PYY and serotonin immunoreactive cells were abundant in the distal portion of the large intestine. Figure 13 shows the ratios of the different kinds of immunoreactive cells to the number of CGA immunoreactive cells. Five of the eight kinds of peptide or serotonin immunoreactive cells constituted 60 to 73 % of endocrine cells in the large intestine other than the rectum where they constituted about 88 %. On the other hand, 84 % of the endocrine cells in the jejunum and 73 % of them in the ileum were composed of endocrine cells with unknown peptides and amines and immature endocrine cells.

The mucosal concentrations of somatostatin in the ascending, transverse, sigmoid colon and the rectum were 25.0 ± 3.9 ng/g, 18.8 ± 3.7 ng/g, 29.3 ± 5.2 ng/g and 87.9 ±27.7 ng/g, respectively (Figure 14). The mucosal concentrations of serotonin in the membrane of the ascending, transverse, sigmoid colon and the rectum were 2011.7 ±281.7 ng/g, 2373.3 ± 696.8 ng/g, 3060.2 ± 473.6 ng/g and 8232.0 ± 2193.0 ng/g, respectively (Figure 15). The mucosal concentrations of somatostatin and serotonin in the rectum were significantly higher than that in the other regions of the large intestine ($p < 0.01$).

Next, immunostaining was performed in 17 cases of rectal carcinoids, the size of which ranged from 0.4 to 1.7 cm. The depth of invasion in all cases was limited to the submucosa and no metastasis to the other organs was

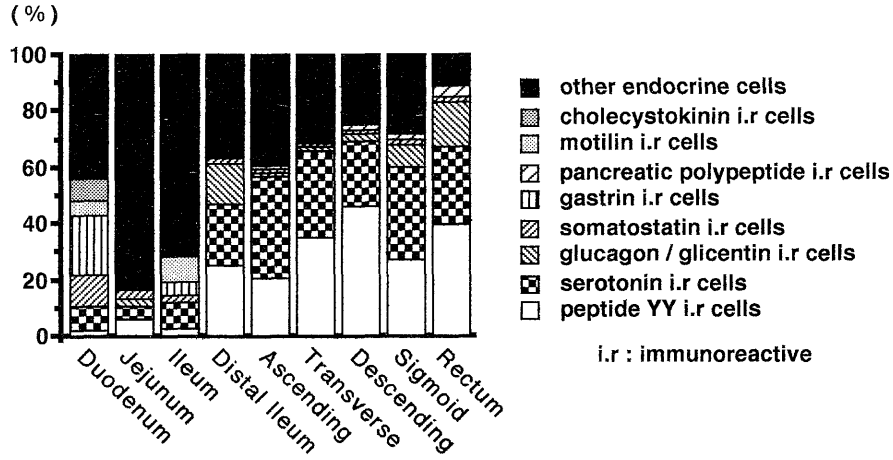

Figure 13. The regional distribution ratio of the different endocrine cell types.

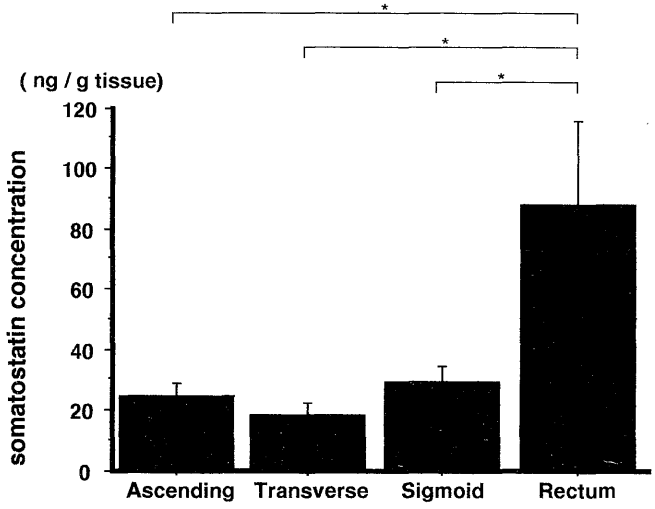

Figure 14. Mucosal concentrations of somatostatin in different regions of colon. ★: $P < 0.01$

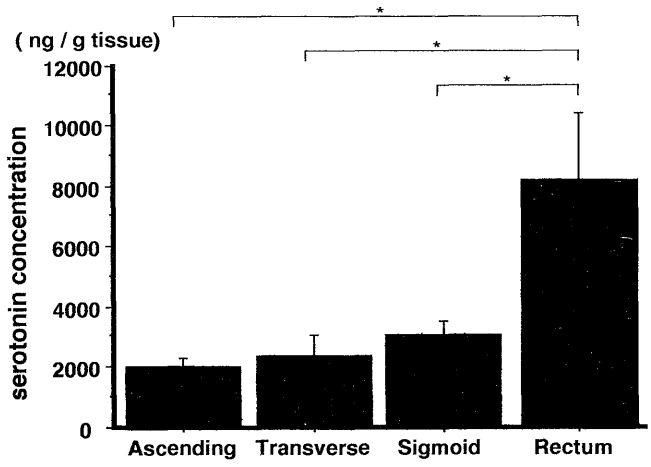

Figure 15. Mucosal concentrations of serotonin in different regions of colon. ★: $P < 0.01$

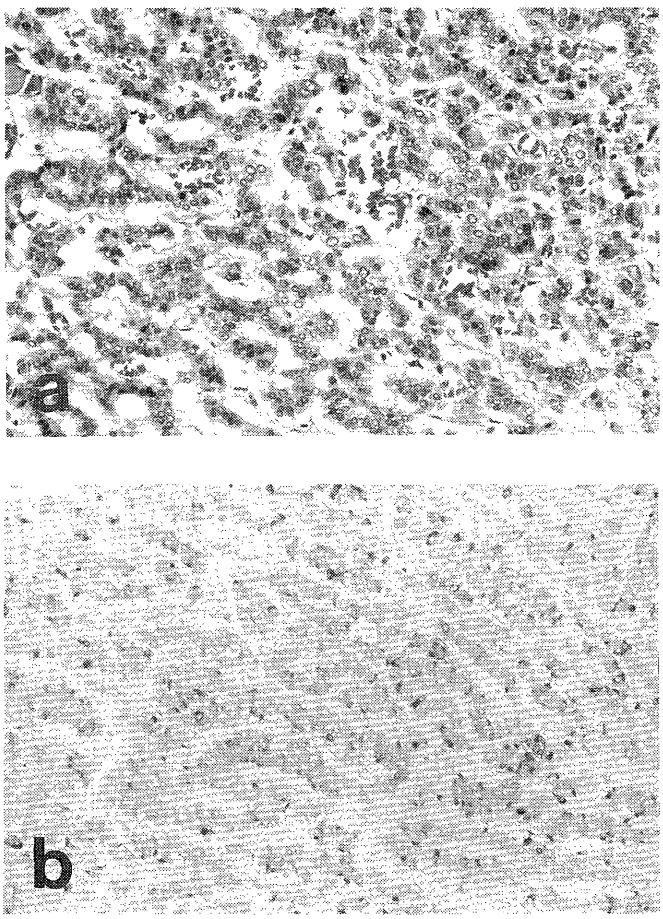

Figure 16 a, b. Rectal carcinoid with trabecular pattern (a). Numerous pancreatic polypeptide immunoreactive cells are found in the tumor cells (b). (a: H&E, X200 b: LAB technique, X200)

Table 4. Details of Patients with Rectal Carcinoid Tumors

No.	Age	Sex	Size (cm)	Depth of Invasion	Histologic type*
1	41	M	0.5	sm	B
2	57	F	0.8	sm	B
3	39	M	0.5	sm	B
4	46	F	1.0	sm	B
5	68	M	0.7	sm	B
6	37	M	0.8	sm	B
7	57	M	1.6	sm	B
8	72	F	1.7	sm	E
9	54	F	0.4	sm	A
10	37	M	0.7	sm	A
11	42	M	1.2	sm	B
12	56	M	0.5	sm	A
13	47	M	0.5	sm	B
14	46	M	1.4	sm	B
15	57	F	1.1	sm	E
16	44	F	1.0	sm	A
17	53	M	0.8	sm	B

*According to Soga's classification A = solid, B = trabecular anastomosing, C = acinar or rosette-like, D = atypical, E = mixed type,

found (Table 4). Histologically, 11 cases were of the trabecular anastomosing type, four cases were of the solid type and two cases were of the mixed type (Figure 16). CGA immunoreactive cells were found in 94% (16/17) of all carcinoids, PP immunoreactive cells in 82% (14/17), PYY immunoreactive cells in 73% (11/15), somatostatin immunoreactive cells in 35% (6/17), and serotonin immunoreactive cells in 12% (2/17). Of the rectal carcinoids examined in this study, 65% (11/17) consisted of cells immunoreactive to two to four kinds of peptides or serotonin (Table 5). However, motilin and gastrin immunoreactive cells which are found only in the normal mucosa of the small intestine were not detected.

Table 5. Immunohistochemical Findings

No.	CGA	PP	PYY	So	Se	Mo	Ga
1	++	++	++	+++	-	-	-
2	+++	-	-	-	-	-	-
3	++	++	+	-	-	-	-
4	++	(+)	ND	-	-	-	-
5	+++	+++	++	-	-	-	-
6	++	++	ND	-	-	-	-
7	++	+	-	-	-	-	-
8	++	++	+	(+)	-	-	-
9	++	++	++	-	-	-	-
10	+++	+	+++	+	++	-	ND
11	+++	+++	++	-	-	-	ND
12	+++	-	-	(+)	-	-	-
13	+++	-	-	-	+++	-	-
14	+++	+	+	+	-	-	-
15	++	+	+	-	-	-	-
16	-	++	++	-	-	-	-
17	++	++	+++	+	-	-	-
Positive (%)	94	82	73	35	12	0	0
Total	(16/17)	(14/17)	(11/15)	(6/17)	(2/17)	(0/17)	(0/15)

CGA : chromogranin A, PP : pancreatic polypeptide, PYY: peptide YY, So: somatostatin, Se : serotonin, Mo : motilin, Ga: gastrin, Grade of positive cell number- : No positive cells, (+) : <1%, + : >1% to 5%, ++ : >5% to 50%, +++ : >50%, ND : not done,

Discussion

In this study, we examined immunohistologically the numbers and kinds of endocrine cells in the normal mucosa of different regions of the small and large intestine. The total number of endocrine cells was determined using an antibody against CGA, a large acidic protein originally found in adrenal medulla and known to exist in endocrine cells throughout the gastro-entero-pancreatic system[4),5),7)]. The evidence has been accumulating that CGA is a precursor of a newly found peptide (pancreastatin) as well as of some other peptides that have already been identified[8),9)]. Moreover, chromogranin is known to be present in all identified endocrine cells in the human intestine[6)], making it a useful specific marker for histochemical detection of endocrine cells, including immature endocrine cells. An antibody against chromogranin therefore enables the determination of the distribution and localization of endocrine cells in the gut mucosa.

CGA immunoreactive cells were present throughout a large region extending from the small intestine to the large intestine. In the small intestine they were more abundant in the proximal portion whereas in the large intestine they were found more frequently in the distal portion, showing the different distribution within the gut. In particular, they were as abundant in the rectum as in the proximal portion of the small intestine. Sjölund et al. estimated the total number of endocrine cells by totaling 15 kinds of peptide or amine immunoreactive cells in different regions of the digestive tract[3)]. The distribution pattern of the endocrine cells deduced from the number of CGA immunoreactive cells determined in this study was similar to that estimated by Sjölund et al.

PYY, serotonin and somatostatin immunoreactive cells were present in a large area extending from the small intestine to the large intestine. PYY and serotonin immunoreactive cells tended to be found more frequently in the distal portion of the large intestine. Glucagon/glicentin immunoreactive cells were found in the region from the jejunum to the rectum, both begin more abundant in the latter. PP immunoreactive cells were absent in the small intestine, and only a small number was found in the large intestine being more abundant in its distal portion. Gastrin, CCK and motilin immunoreactive cells were found only in the proximal portion of the small intestine.

We examined the correlation between the physiological effects of the secreted peptides and amines and their distribution. The main physiological effects of gastrin are stimulation of hydrochloric acid secretion from the parietal cells of the stomach, stimulation of secretion of pepsin and the intrinsic factor[10)], stimulation of mucosal blood flow in the stomach, and the trophic effect on the gastrointestinal tract[11)]. CCK enhances the contraction of the gallbladder and the secretion of the pancreatic enzymes, inhibits gastric emptying[12)], stimulates pancreatic growth[13),14)], and induces PP secretion[15),16)]. The physiological effects of motilin include stimulation of type Ⅲ myoelectric activity in the stomach and duodenum and stimulation of contraction of the smooth muscles in the small intestine, the gallbladder, the sphincters of Oddi and the lower esophageal sphincters[17)]. That is, the endocrine cells secreting these three peptides are located near their target organs. Glucagon/glicentin immunoreactive cells were present in the region from the jejunum to the rectum. Glicentin (enteroglucagon) has been reported to affect the intestine in defferent ways such as inhibition of intestinal motility[18),19)], stimulation of blood flow[19)] and induction of epithelial cell proliferation[18),19)]. However the knowledge about its action is limited, and it seemed difficult to correlate the distribution of glucagon/glicentin immunoreactive cells with a physiological action.

Somatostatin, PYY and serotonin immunoreactive cells were found in a large region extending from the small intestine to the large intestine. The diverse effects of somatostatin, such as inhibition of secretion of many gut peptides as well as gastric and intestinal juice[20),21)], inhibition of intestinal blood flow and nutrient absorption[20),21)], control of the gastrointestinal motility, the prolongation of gastric emptying and intestinal transit time, and inhibition of gallbladder contraction[21)], may be related to the wide distribution of the somatostatin immunoreactive cells. Serotonin and PYY immunoreactive cells were present in both the small intestine and large intestine, being the most abundant in the rectum. Serotonin relaxes the intestinal anal sphincters in rat[22)]. The serotonin concentration in the mucosa and circular muscle of the sigmoid colon have been reported to be increased in patients with severe idiopathic constipation[23)]. PYY has also been reported to inhibit the motility of the large intestine[24)] and PYY producing ovarian carcinoids to cause severe constipation[25)]. Therefore, the presence of a large number of serotonin and PYY immunoreactive cells in the distal portion of the large intestine, such as the sigmoid colon and the rectum, was suggested to be related to the suppression of motility of the distal side of the large intestine.

We examined the composition of the endocrine cells in different regions of the small and large intestine. Since the eight kinds of endocrine cells examined in this study constituted only a small portion of the total endocrine cells within the small intestine, particularly in the jejunum and ileum, there may be many endocrine cells of unknown types, immature endocrine cells, or endocrine cells not examined in this study. On the other hand, the eight kinds of endocrine cells were abundant in the large intestine ; in the rectum in particular, they together accounted for about 88 % of the total endocrine cells. Therefore unknown or immature endocrine cells were in a small number in the rectum. Recently, there has been an increasing number of

reports showing that two or more kinds of peptides and amines coexist in one immunoreactive cell[26),27)]. In this study, we did not take this possibility into consideration. In further studies it will be necessary to solve this problem of coexistence to investigate the accurate composition of the endocrine cells in different regions of the small and large intestines.

Recently, it has become possible to measure mucosal concentrations of gut hormones. We also measured the mucosal concentrations of somatostatin and serotonin in each region of the large intestine and examined the correlation between their concentrations and the numbers of endocrine cells. The mucosal concentrations of somatostatin and serotonin were both high in the distal portion of the large intestine, particularly in the rectum. The mucosal concentrations of somatostatin and serotonin in the rectum were significantly higher than those in the other regions of the large intestine. Our findings were similar to those obtained in the studies on the mucosal concentrations of somatostatin[28)] and serotonin[29)] in biopsy specimens of the large intestine. The spatial pattern of the mucosal concentrations of somatostatin and serotonin in the large intestine approximately coincided with that of the numbers of somatostatin and serotonin immunoreactive cells. This finding shows a correlation between the mucosal concentration and numbers of these endocrine cells.

We examined 17 cases of rectal carcinoid immunohistochemically. 94% of the cases (16 out of 17 cases) were immunoreactive to CGA, and 11 out of 17 cases were immunoreactive to more than two peptides or amine. PP and PYY immunoreactive cells were present at high frequencies, 82% and 73%, respectively, whereas somatostatin and serotonin immunoreactive cells were detected in 35% and 12% of the cases, respectively. The cells immunoreactive to peptides and amine such as PP, PYY, somatostatin and serotonin found in the rectal carcinoids were also found in the normal rectal mucosa examined in this study. None of the 17 cases of rectal carcinoids were positive to the peptides that were not detected in the normal rectal mucosa. The peptides and amines detected in the gastrointestinal carcinoid are reported to be mostly those normally present in the regions where the carcinoids develop[30),31)], and our findings support this view.

Although there are minor differences among the reports, the preferred sites of gastrointestinal carcinoids found in autopsy in Japan are, in the decreasing order, the stomach, rectum, duodenum, and ileum[32)]. In this study, the regions where carcinoids occurred frequently in the small and large intestines corresponded to the regions rich in CGA immunoreactive cells.

The precursor cells of the carcinoid are thought to be derived from immature endocrine cells and not stem cells capable of differentiating into various cell types[33),34)]. In this study, we found a large number of endocrine cells with unknown hormones and immature endocrine cells in the small intestine, whereas these cells were scarce in the large intestine, particularly in the rectum. That is, the rectum, one of the regions where gastrointestinal carcinoids occur most frequently, was not heavily populated with immature endocrine cells. Therefore, the frequent occurrence of carcinoids in the rectum cannot be attributed to the number of immature endocrine cells. It will be necessary to take into consideration the difference in the renewal rate of endocrine cells between the rectum and the other regions of intestine and other background factors.

Ackmowledgement

The authors would like to thank Prof. Kouhei Hara, the Second Department of Internal Medicine, Nagasaki University School of Medicine for his advice and review of the manuscript. Thanks are also due to Prof. Ichiro Sekine, Department of Pathology, Anatomic Disease Institute, Nagasaki University School of Medicine for valuable suggestions.

References

1) Fujita T., and Kobayashi S.: The cells and hormones of the GEP endocrine system -the current of studies. In Fujita T (ed): Gastro-Entero-Pancreatic System. A Cell Biological Approach., 1-16, Igaku-Shoin, Tokyo, 1973.
2) Fujita T., and Kobayashi S.: The endocrine cell. In Bloom SR, Polak JM (eds): Gut Hormones 2nd ed., 90-95, Churchill Livingstone, Edinburgh, 1981.
3) Sjölund K., Sanden G., Kakanson R., and sundler F.: Endocrine cells in human intestine: An immunocytochemical study. Gastroenterology 85: 1120-1130, 1983.
4) Wilson BS., and Lloyd RV.: Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. Am J Pathol 115: 458-468, 1984.
5) Cetin Y., Muller-Koppel L., Aunis D., Bader M-F., and Grude D.: Chromogranin A (CgA) in the gastro-entero-pancreatic (GEP) endocrine system. II. CgA in mammalian entero-endocrine cells. Histochemistry 92: 265-275, 1989.
6) Facer P., Bishop AE., Lloyd RV., Wilson BS., Hennessy RJ., and Polak JM.: Chromogranin: a newly recognized marker for endocrine cells of the human gastrointestinal tract. Gastroenterology 89: 1366-1373, 1985.
7) Wiedenmann B., and Huttner WB.: Synaptophysin and chromogranins/secretogranins-widespread constituents of distinct types of neuroendocrine vesicles and new tools in tumor diagnosis. Virchows Arch [B] 58: 95-121, 1989.
8) Tatemoto K., Efendic S., Mutt V., Makk G., Feistner GJ., and Barchas JD.: Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature 324: 476-478, 1986.
9) Huttner WB., and Benedum UM.: Chromogranin A and pancreastatin. Nature 325: 305, 1987.
10) Eysselein VE., Maxwell V., Reedy T., Wunsch E., and Walsh JH.: Similar acid stimulatory potencies of synthetic human big and little gastrins in man. J Clin Invest 73: 1284-1290, 1984.
11) Johnson LR.: New aspects of the trophic action of gastrointestinal hormones. Gastroenterology 72: 788-792, 1977.
12) Kleibeuker JH., Beekhuis H., Jansen JB., Piers DA., and Lamers CB.: Cholecystokinin is a physiological hormonal mediator of fat-induced inhibition of gastric emptying in man. Eur J Clin Invest 18: 173-177,

1988.
13) Niederau C., Liddle RA., Williams JA., and Grendell JH.: Pancreatic growth: Interaction of exogenous cholecystokinin, a protease inhibitor, and a cholecystokinin receptor antagonist in mice. Gut 28 supple: 63-69, 1987.
14) Winsner JR Jr., McLaughlin RE., Rich KA., Ozawa S., and Runner IG: Effect of L-364, 718, a new cholecystokinin receptor antagonist, on camostate-induced growth of the rat pancreas. Gastroenterology 94: 109-113, 1988.
15) Lonovics J., Guzman S., Devitt P., Hejitmancik KE., Suddith RL., Rayford PL., and Thompson JC.: Release of pancreatic polypeptide in humans by infusion of cholecystokinin. Gastroenterology 79: 817-822, 1980.
16) Hosotani R., Chowdhury P., and Rayford PL.: L-364, 718, a new CCK antagonist, inhibits postprandial pancreatic secretion and PP release in dogs. Dig Dis Sci 34: 462-467, 1989.
17) Brown JC., Cook MA., and Dryburgh JR.: Motilin, a gastric motor activity-stimulating polypeptide. Gastroenterology 62: 401-404, 1972.
18) Bloom SR.: An enteroglucagon tumor. Gut 13: 520-523, 1972.
19) Gleeson MH., Bloom SR., Polak JM., Henry K., and Dowling RH.: Endocrine tumor in kidney affecting bowel structure, motility and absorptive function. Gut 12: 773-782, 1971.
20) Arnold R., and Lankisch PG.: Somatostatin and the gastrointestinal tract. Clin Gastroenterol 9: 733-753, 1980.
21) Newman JB., Lluis F., and Townsend CM Jr.: Somatostatin. In Thompson JC, Greeley GH Jr, Rayford PL, et al (eds): Gastrointestinal Endocrinology., 286-299, McGraw-Hill Book Co., New York, 1987.
22) Goldberg M., Hanani M., and Nissan S.: Effects of serotonin on the internal anal sphincter: in vivo manometric study in rats. Gut 27: 49-54, 1986.
23) Lincoln L., Crowe R., Kamm MA., Burnstock G., and Lennard-Jones JE.: Serotonin and 5-hydroxyindoleacetic acid are increased in the sigmoid colon in severe idiopathic constipation. Gastroenterology 98: 1219-1225, 1990.
24) Sawa T., Mameya S., Yoshimura M., Itsuno M., Makiyama K., Niwa M., and Taniyama K.: Differential mechanism of peptide YY and neuropeptide Y in inhibiting motility of guinea pig colon. In press, 1994.
25) Motoyama T., Katayama Y., Watanabe H., Ozaki E., and Shibuya H.: Functioning ovarian carcinoids induced severe constipation. Cancer 70: 513-518, 1992.
26) Ali-Rachedi A., Varndell IM., Adrian TE., Gapp DA., Van Noorden S., Bloom SR., and Polak JM.: Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. Histochemistry 80: 487-491, 1984.
27) Bottcher G., Sjölund K., Ekblad E., Hakanson R., Schwartz TW., and Sundler F.: Co-existence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. Regul Pept 8: 261-266, 1984.
28) Calam J., Ghatei MA., Domin J., Adrian TE., Myszor M.,Gupta S., Tait C., and Bloom SR.: Regional differences in concentrations of regulatory peptides in human colon mucosal biopsy. Dig Dis Sci 34: 1193-1198, 1989.
29) Takemasa Y.: Serotonin concentrations and its turnover index in biopsied human colonic tissues. Gastroenterol Endosc 35: 3-8, 1993. (in Japanese)
30) Iwafuchi M., Watanabe H., Noda Y., Ajioka Y., Enjoji M., and Ito S.: Gastrointestinal carcinoid tumors of Japanese: Incidence and characteristics based on anatomical classification, with special reference to difference between carcinoid tumor and endocrine cell carcinoma. I to Cho 24: 868-882, 1989. (in Japanese with English abstract)
31) Dayal Y.: Endocrine cells of the gut and their neoplasms. In H T Norris (ed): Contemporary Issues in Surgical Pathology. Vol. IV., 267-302, Churchill Livingston, New York, 1993.
32) Soga J., and Suzuki T.: Carcinoids and the carcinoid syndrome. Nippon rinsho 650: 207-221, 1993.
33) Soga J.: Histogenesis of carcinoid tumor. I to Cho 10: 625-633, 1975. (in Japanese with English abstract)
34) Iwafuchi M., Watanabe H., Ishihara N., Ishihara N., Enjoji M., Iwashita A., Yanaihara N., and Ito S.: Neoplastic endocrine cells in carcinomas of the small intestine: Histochemical and immunohistochemical studies of 24 tumors.: Hum Pathol 18: 185-194, 1987.