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Serotonin-containing EC cells in normal human gastric mucosa and in gastritis

Immunohistochemical, electron microscopic and autoradiographic studies

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Summary. Serotonin-containing EC cells in human fetal, infantile and adult stomachs both normal and affected by gastritis, were studied by immunohistochemical, electron microscopic and autoradiographic methods. EC cells were sparse in fetal and infantile stomachs, while they occurred in the lower half of the gastric mucosa in adult stomachs showing no atrophic changes and their distribution density was higher than that of D cells. With the progress of chronic gastritis, the number of EC cells gradually decreased, but intestinal type of EC cells appeared in intestinalized gastric mucosa, often showing hyperplasia. Most of EC cells showed argyrophil reaction, but only about 10–20% of them were positive with argentaffin. Epithelial cells with ^{3}H-TdR labeled nuclei were frequently detected in the gastric mucosa where EC cells were sparse or almost absent. Electron microscopically, EC cells had typical electron dense granules in both the normal gastric mucosa and in the intestinal metaplastic glands, but the number of secretory granules was greater in the latter than in the former. These findings suggested that EC cells are preferentially present in the gastric mucosa with a small number of labeled nuclei and have morphological heterogeneity.

Key words: Serotonin – EC cells – Human stomach – Immunohistochemistry

Introduction

Serotonin-containing enterochromaffin cells (EC cells) are distributed throughout the gastrointestinal mucosa and store about 90% of the total body product (Resnick and Gray 1961). EC cells can be demonstrated by

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the formaldehyde-induced fluorescence (FIF) technique on a freeze dried section (Facer et al. 1979; Nemoto et al. 1982) as first reported by Falck et al. (1962). Because of the difficulties involved in FIF techniques, such as tissue preservation or rapid decline of the specific fluorescence, few reports have been made on the distribution of EC cells in human gastric mucosa in normal and gastritis affected stomachs. However, recent advances in immunohistochemical procedures and production of specific antibody to serotonin have enabled demonstration of EC cells even in paraffin sections.

The purpose of this study was to examine the distribution and cell kinetics of EC cells in normal human gastric mucosa and gastritis and to clarify the pathophysiological role of EC cells in comparison with that of other types of endocrine cells.

Materials and methods

Studies were made on 14 surgically resected stomachs composed of four cases with gastric carcinoma, eight with gastric adenoma and two with peptic ulcer. Six fetal (4, 5, 6, 7, 8 and 10 months of gestation) and one infantile (3 months after birth) stomachs were obtained by therapeutic abortion or at autopsy within 3 h after death. They were fixed in 10% formalin or Bouin's solution and embedded in paraffin wax. Four to six representative blocks were taken from each case for light microscopy and immunohistochemistry. In two cases with peptic ulcer the entire resected stomach was examined by making longitudinal strips, 4 mm in width, parallel to the lesser curvature. Four cases with gastric carcinoma were also subjected to autoradiographic procedure.

Adjacent or serial sections were cut 4 μm in thickness and were stained with haematoxylin and eosin, Grimelius silver nitrate technique for argyrophil reaction and Fontana-Masson's silver impregnation method for argentaffinity.

We regarded gastric mucosa with no atrophy and only slight inflammatory cells in lamina propria as "normal" in this article.

Immunohistochemistry. For the detection of serotonin in gastric mucosa, the avidin-biotin-peroxidase complex (ABC) method after Hsu et al. (1981) was carried out. The unlabeled antibody method using PAP complex after Sternberger (1979) was applied for the detection of gastrin, somatostatin, glicentin and motilin. Incubation with antibodies or rinsing in PBS after each step was performed at least for 30 min at room temperature. Endogenous peroxidase activity was inactivated by immersing the specimens in 0.03% hydrogen peroxide in absolute methanol for 20 min. The sections were counterstained with 3% methyl green.

Anti-serotonin rat monoclonal antibody was purchased from Sera-Lab, England, and employed at a 1:600 dilution. Preparation and characterization of glicentin (R4804) and gastrin antiserum have been described previously (Yanaihara 1980; Tahara et al. 1982; Ito et al. 1984). Antibody for somatostatin was purchased from Japan Immunoresearch Laboratories Co. Ltd., and lyophilized preparation was diluted 1:150. Anti-motilin serum was obtained from Cambridge Research Biochemical, USA and diluted 1:400. Anti-rabbit IgG was prepared by MBL Company, Japan and absorbed by human immunoglobulin without known cross-reactivities against other animal proteins. Peroxidase-antiperoxidase complex (rabbit) was obtained from Dakopatts and diluted 1:100. Biotinylated anti-rat IgG and avidin-biotinylated horse radish peroxidase complex (ABC) were purchased from Vector Laboratories, Inc, CA, USA. Serotonin-creatinine sulfate complex was purchased from WAKO Pure Chemical Industries, Ltd, Japan.

To examine the specificity of immunostaining for serotonin, the antiserotonin antibody was replaced by 1) normal IgG or 2) anti-serotonin antibody previously absorbed with serotonin-creatinine sulfate complex (5 and 50 μg/ml diluted antibody). Control slides were invariably negative.

Electron microscopy. Small tissues of fundic and antral mucosa were obtained immediately after operation and fixed in Zamboni solution for 1–7 days followed by 1% osmium tetroxide for 4–15 min, dehydrated and embedded in Epon 812. A semithin section from Epon embedded tissue was placed on a glass microscope slide coated with 1% gelatin. After removal of plastic with saturated potassium hydroxide, the section was subjected to immunostaining for serotonin as described above. Serial thin sections were double-stained with uranyl acetate and lead citrate, and then examined with a JEM 1200EX electron microscope.

Autoradiographic procedure. Ex vivo autoradiography as reported by Nakamura et al. (1983) was performed in four cases of gastric carcinoma. The surgically resected stomach was quickly brought from the operating room to the RI Center. The left and right gastric arteries were cannulated and perfused by 200 ml physiological saline with heparin. It was then put into an aluminium box filled with physiological saline at 37° C and perfused with 600 ml of Fluosol-DA 20% (FDA) (The Green Cross Corp., Osaka, Japan), containing 1,000 μCi equivalent of tritiated thymidine (^{3}H-TdR) (New England Nuclear, Boston, Mass., USA, specific activity, 20 Ci/mmol). FDA was previously oxygenated to obtain a partial pressure of oxygen of 200 mm Hg. The perfusion time ranged from 40 to 60 min. Soon after perfusion with FDA containing ^{3}H-TdR, the stomach was reperfused with 100 ml of physiological saline to wash out the residual isotope in the vessels. They were then fixed in 10% buffered formalin and embedded in paraffin wax as usual. The deparaffinized sections were dipped in Sakura NR-M2 emulsion (Konishiroku Photo Ind. Co., Ltd.), stored at 4° C for four weeks in a dark box, developed and counter-stained with nuclear fast red.

Results

In fetal and infantile stomachs, a good number of gastrin-containing G cells (Fig. 1a) and somatostatin-containing D cells were detected in the an-

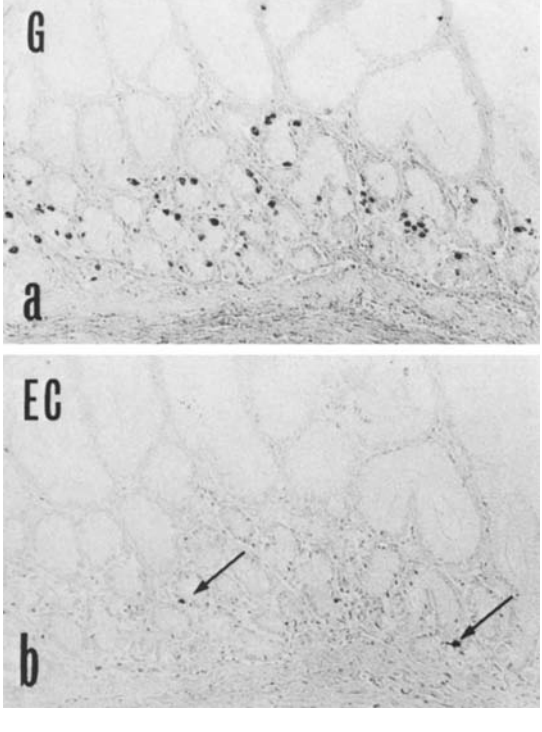

Fig. 1a, b. Serial sections from fetal antral mucosa (8 months of gestation.
(**a**) A good number of gastrin-containing G cells are detected in the lower half of the gastric mucosa. Immunostaining with anti-gastrin antibody (×120).
(**b**) Only a few serotonin-containing EC cells (*arrows*) were found in the gastric antral mucosa. Immunostaining with anti-serotonin antibody. (×120)

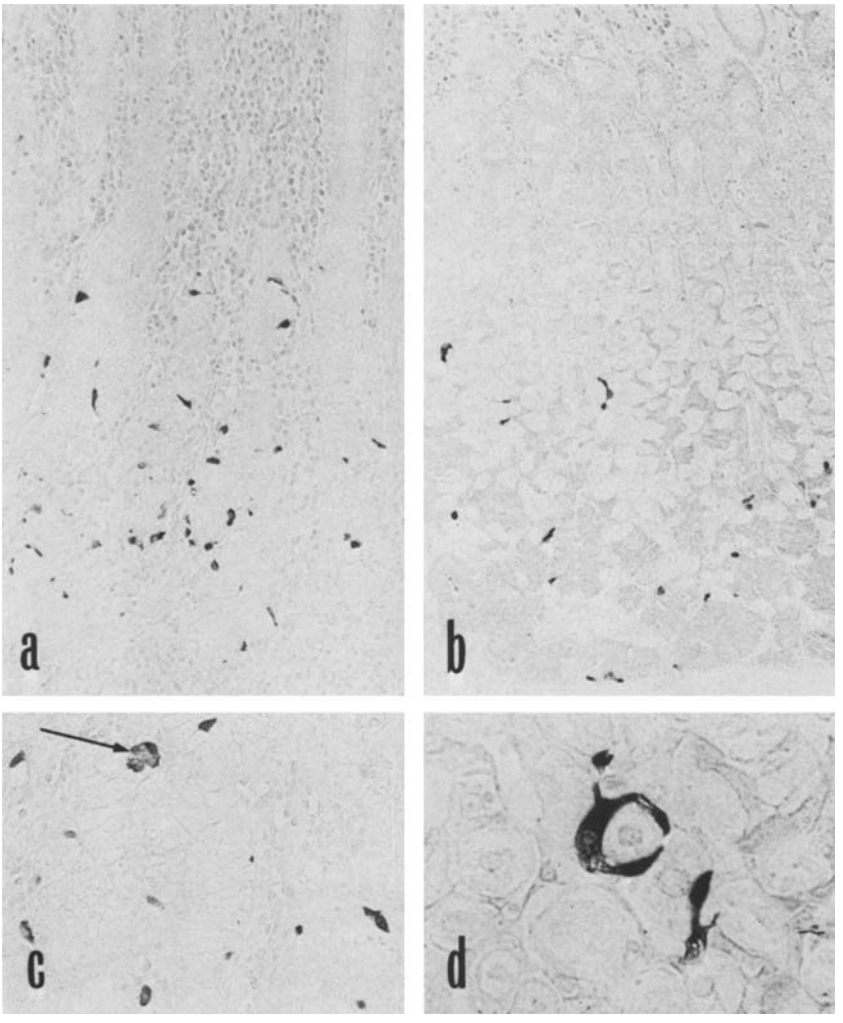

Fig. 2a–d. Serotonin-containing EC cells in normal adult stomach. (**a**) A fairly good number of EC cells are distributed in the lower half of the antral mucosa (×140). (**b**) EC cells are sparsely distributed in fundic mucosa (×140). (**c**) EC cells in the antral mucosa with round or oval appearance. Micronodule of EC cells (*arrow*) is also noted (×235). (**d**) EC cells in the fundus show closed type character and occasionally envelop parietal cells (×450)

tral mucosa. Only a few isolated serotonin-containing EC cells (Fig. 1b) were sparsely scattered in the antral and fundic mucosa. The ratio of EC cells to G or D cells in the antral mucosa was about 1:50–100

In portions of adult stomach showing no atrophic changes, EC cells were distributed in the lower half of the gastric mucosa in both the antrum and fundus (Fig. 2a, b). EC cells, which showed comma-like or round appearance, sometimes had direct contact with the gland lumen in the antrum (Fig. 2c). However, EC cells, which were oval to fusiform, had no direct

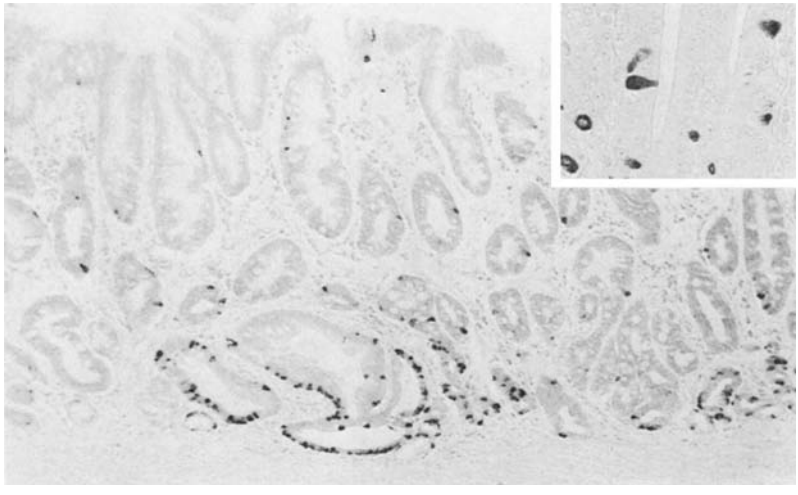

Fig. 3. EC cells in the gastric mucosa with intestinal metaplasia. They are irregularly distributed with a hyperplastic appearance (×80). (*Insert:* EC cells in metaplastic glands show open cell character with direct contact with the gland lumen. ×230)

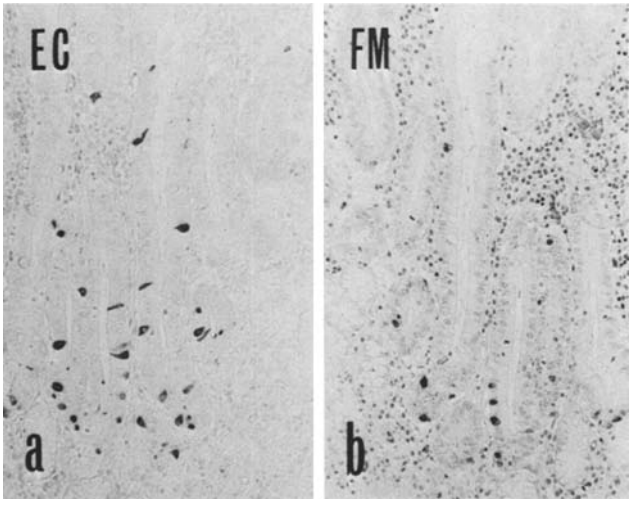

Fig. 4. A pair of mirror sections from the antral mucosa with intestinal metaplasia. (**a**) A large number of EC cells are noted in metaplastic glands. Immunostaining with anti-serotonin antibody (×115). (**b**) Only a few EC cells show argentaffinity. Fontana-Masson's silver impregnation (×115)

contact with the lumen and occasionally surrounded parietal cells in the fundus (Fig. 2d). The distribution density of endocrine cells ranged in the order of G cells > EC cells > D cells in the antrum and fundus, which had less than mild atrophic gastritis. With the progress of chronic atrophic gastritis, these endocrine cells gradually decreased and micronoduli of EC cells

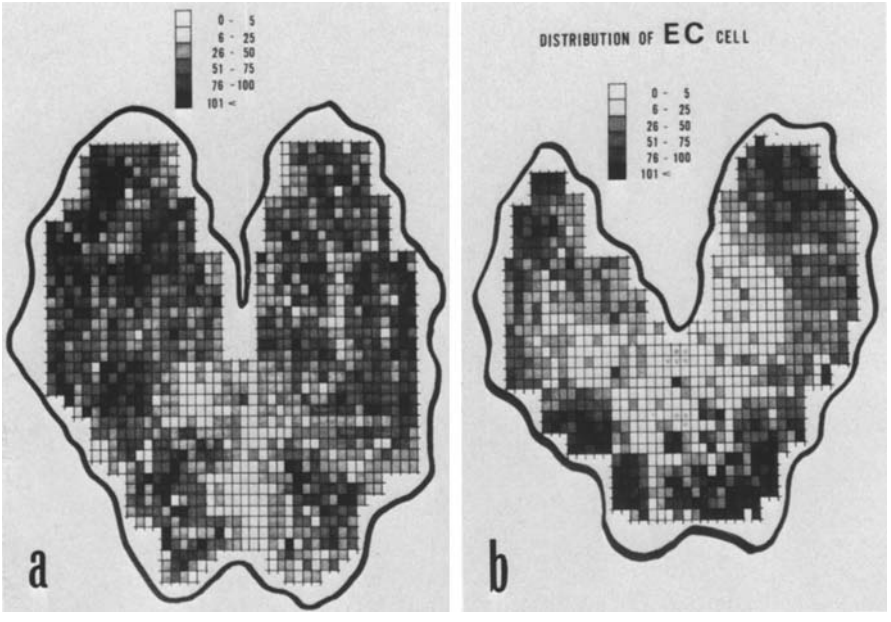

Fig. 5. Whole gastric map on the distribution of EC cells. (**a**) Duodenal ulcer in a 37 year old male. (**b**) Gastric ulcer in a 65 year old male

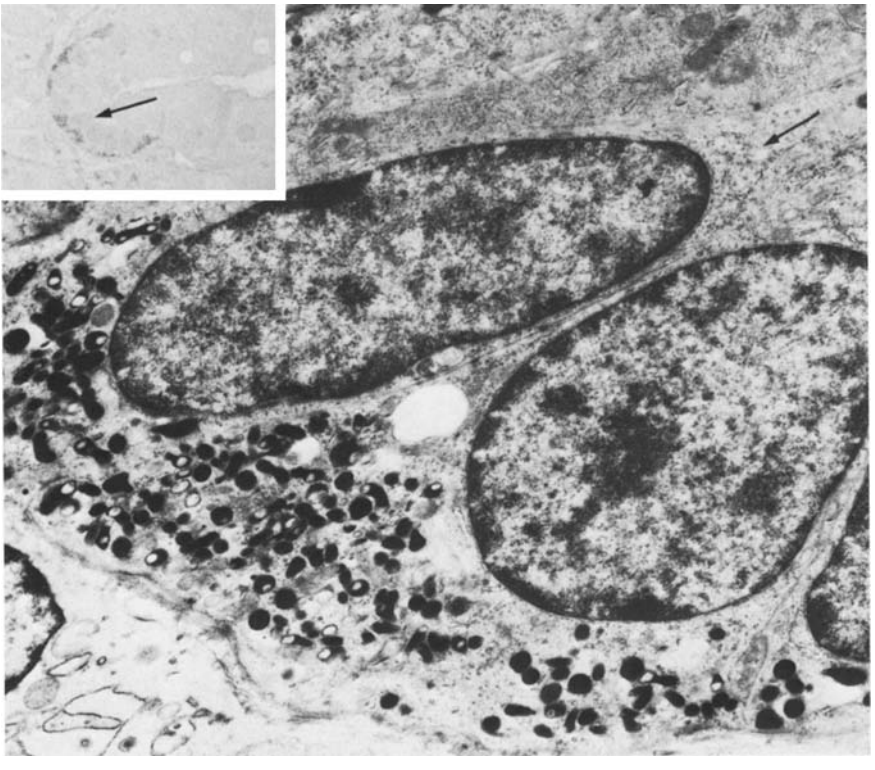

Fig. 6. Electron microscopic appearance of EC cells in a metaplastic gland. A good number of round or elongated secretory granules (from 150 to 350 diameter) are found in the basal region of the cells (× 8,100). (*Insert:* Immunostaining with anti-serotonin antibody in semi-thin section. The same cell is indicated by *arrow*. × 410)

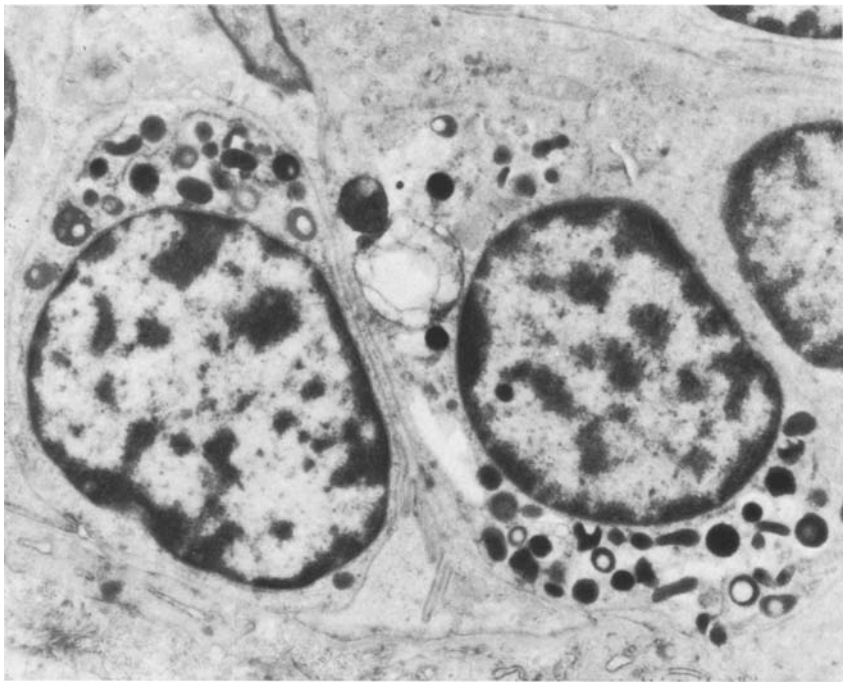

Fig. 7. Electron microscopic appearance of EC cells in normal antrum. The number of secretory granules is smaller than that of intestinal metaplastic glands (× 8,100)

occasionally occurred in the lamina propria (Fig. 2c). EC cells did not appear in the epithelial lining of regenerative cells around the gastric ulcer.

EC cells appeared most commonly in the deeper zone of the intestinalized gastric mucosa and showed uneven distribution (Fig. 3). They showed a columnar appearance and had obviously open cell character with direct contact with the gland lumen (Fig. 3 insert). Glicentin-containing L cells frequently occurred in metaplastic glands, associated occasionally with hyperplastic appearance in the deeper zone of the gastric mucosa, as described in detail elsewhere (Ito et al. 1984). Hyperplasia of EC cells, however, was found in completely intestinalized glands, whereas L cells were sparse in completely metaplastic glands. A few motilin-containing Mo cells were occasionally detected in metaplastic glands, but coexistence of motilin and serotonin immunoreactivity was not observed in the same cell.

Most of EC cells in the gastric mucosa showed argyrophil reaction using Grimelius silver nitrate. On the other hand, argentaffinity by Fontana-Masson's techniques was demonstrated in a few EC cells and was estimated to be about 10–20% (Fig. 4a, b).

In two cases with duodenal and gastric ulcer, the entire resected stomach was cut totally and prepared for immunohistochemical procedure to investigate the distribution of EC cells (Fig. 5a, b). Histological findings and distribution of the G, D and calcitonin-containing C cells in these cases have

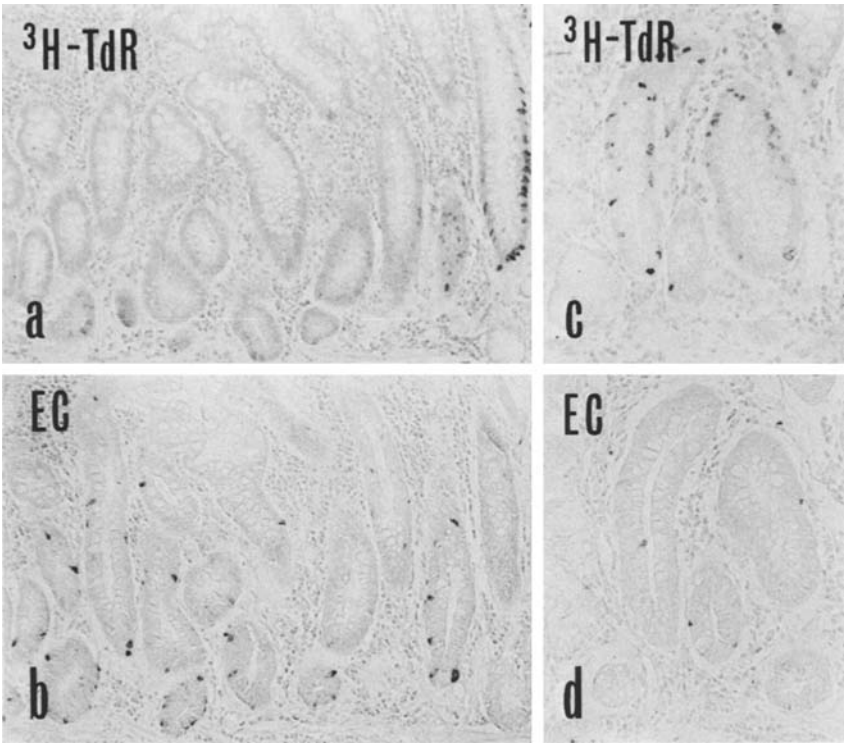

Fig. 8. Distribution of epithelial cells with ^{3}H-TdR labeled nuclei and EC cells in intestinal metaplastic glands. (**a**) A few epithelial cells with labeled nuclei are seen in completely intestinalized mucosa. Ex vivo autoradiography (× 85). (**b**) EC cells, which are diffusely distributed, are not associated with labeled nuclei having cells. Semi-serial section of photograph **a**. Immunostaining with anti-serotonin antibody (× 85). (**c**) Metaplastic glands with numerous epthelial cells with labeled nuclei. Ex vivo autoradiography (× 130). (**d**) Only a few EC cells are found in the metaplastic glands having a good number of cells with labeled nuclei. Semi-serial section of photograph (**c**) (× 130)

been previously reported (Ito and Tahara 1983; Ito et al. 1986). There was no significant difference in the distribution density of EC cells between the antrum and fundus. Because of the presence of chronic atrophic gastritis without intestinal metaplasia, the distribution density of EC cells was significantly lower in the lesser curvature than in the greater curvature in the antrum of duodenal ulcer (Fig. 5a). In case of gastric ulcer, the number of G and D cells markedly decreased in the antrum (Ito and Tahara 1983), but EC cells showed a rather higher distribution density because of the occurrence of intestinal type EC cells in metaplastic glands (Fig. 5b).

Electron microscopically, a good number of characteristic round to elongated intracytoplasmic granules ranging 150–350 nm in diameter (Figs. 6, 7) were demonstrated in the serotonin-containing EC cells using serial thin and semi-thin techniques. Secretory granules of EC cells in metaplastic glands were localized at the basal region of these cells (Fig. 6). In normal

antral and fundic mucosa, they were distributed diffusely in the cytoplasm of EC cells (Fig. 7). The number of secretory granules was apparently greater in the former.

In ex vivo autoradiography, only areas with successful ^{3}H-TdR up-take sufficient for the study of cell kinetics were examined and evaluated. In normal gastric mucosa, labeled nuclei were detected in the neck zone of the fundus and in the middle third of the antrum where G cells were distributed. With the progress and extension of chronic atrophic gastritis, labeled nuclei were localized to the deeper area of the gastric mucosa and the number of EC cells decreased in these mucosae. The proliferative zone in intestinal metaplasia was observed at the bottom of the gland. In a completely intestinalized gastric mucosa with hyperplastic EC cells, only a few labeled nuclei were observed (Fig. 8a, b). Few EC cells were found in glands or mucosa with a good number of labeled nuclei (Fig. 8c, d). There was a reversed correlation between the distribution of EC cells and labeled nuclei.

Discussion

It is well accepted that enterochromaffin cells (EC cells) in the gastrointestinal tract are roughly identical with argentaffin cells (Dayal 1983; Mitschke and Becker 1973; Tsutsumi et al. 1983). Argentaffin cells have been shown to develop more frequently in the gastric mucosa of chronic gastritis, intestinal metaplasia or gastric cancer than in normal gastric mucosa (Eklof 1914; Hamperl 1927; Mitschke and Becker 1973; Singh 1966). However, in this study only 10–20% of EC cells showed argentaffinity, although the majority of them were argyrophil. Therefore, compared with the number of EC cells reported previously, more EC cells are distributed in the gastric mucosa in normal or gastritis stomachs. Moreover, EC cells had a higher density than that of somatostatin-containing D cells.

While the epithelial cells with ^{3}H-TdR labeled nuclei and the gastrin-containing G cells showed a similar distribution in the middle third of the normal antral mucosa, EC cells were more widely distributed, being located in the deeper zone of the antral mucosa. Moreover, EC cells occasionally showed hyperplasia in the metaplastic glands where the epithelial cells with labeled nuclei were sparse or almost absent. These findings suggest that EC cells are well differentiated and localized in the gastric mucosa with a stable condition remote from the proliferative zone. This speculation might be supported by the fact that EC cells were scattered or almost absent in fetal gastric mucosa or in the epithelial lining of regenerative cells around the gastric ulcer.

EC cells have been subdivided into EC_1, EC_2 and EC_n according to the ultrastructural and immunohistochemical characteristics (Solcia et al. 1978). EC_1 cells (intestinal type EC cells) contain leu-enkephalin and substance P, and EC_2 cells (duodenal type EC cells) have leu-enkephalin and motilin-like peptide. The peptide(s) stored in EC_n cells (gastric type EC cells) have not yet been elucidated. In the present study, the coexistence of motilin and serotonin immunoreactivity in the same cell was not found

even in metaplastic glands. Tsutusmi et al. (1983) have also failed to demonstrate substance P-containing cells in the gastric mucosa. Moreover, EC cells were unevenly distributed and occasionally showed hyperplasia in intestinalized gastric mucosa, whereas in the small intestine they were sparsely and regularly distributed. Therefore, intestinal metaplasia of the stomach does not seem to display mere replacement of the gastric mucosa by the mucosa of the small intestine. In particular, there are evident differences in the distribution ratio of endocrine cells between the intestinalized gastric mucosa and the small intestine, as reported by Tsutsumi et al. (1983).

Electron microscopically, the characteristic secretory granules for EC cells were successfully demonstrated in serotonin-containing cells by immunohistochemical techniques. This might be indirect morphological evidence of the specificity of the anti-serotonin monoclonal antibody used in the present study. The intracellular distribution and number of secretory granules were different between EC cells in the antrum or fundus and EC cells in the metaplastic gland. These differences might reflect the morphological heterogeneity of EC cells.

In view of the results of the present study on the distribution and heterogenity of EC cells in the stomach, the role of EC cells may be not necessarily limited to the vasoconstrictor action of serotonin. Saik (1981) reported that serotonin inhibited basal gastrin output and discussed the effect of serotonin in preventing ulcerative diseases. In this study, EC cells clearly showed a distribution different from those of G cells and glicentin-containing L cells having a trophic actions. Moreover, a paracrine relationship between EC cells and parietal cells was also suggested. EC cells may therefore play an important role in not only the motility of the gut but also in the inhibition of gastrin and gastric acid secretion. The interaction of EC cells with other endocrine cells such as D or L cells remains to be investigated.

References

Dayal Y (1983) Endocrine cells of the gut and their neoplasms. In: Norris HT (ed) Pathology of the Colon, Small Intestine and Anus. Churchhill Livingstone, New York, Edinburgh and Melbourne, pp 267–302

Eklof H (1914) Chondriosomenstudien an den Epithel- und Drüsenzellen des Magen-Darmkanals und Ösophagusdrüsenzellen bei Säugetieren. Anat Hefte 51:1–227

Facer P, Polak JM, Jaffe BM, Pearse AGE (1979) Immunohistochemical demonstration of 5-hydroxytryptamine in gastrointestinal endocrine cells. Histochem J 11:117–121

Falck B, Hillarp NA, Thieme G, Torp A (1962) Fluorescence of catechol amines and related compounds condensed with formaldehyde. J Histochem Cytochem 10:348–354

Hamperl H (1927) Über die „gelben (chromaffinen)" Zellen im gesunden und kranken Magendarmschlauch. Virchows Arch [Pathol Anat] 266:509–548

Hsu SM, Raine L, Fanger H (1982) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Ito H, Tahara E (1983) Immunohistochemical study on G and D cells in human resected stomach with peptic ulcer diseases. In: Miyoshi A (ed) Gut peptides and ulcer. Biomedical Research Foundation. Tokyo, pp 180–187

Ito H, Yokozaki H, Hata J, Mandai K, Tahara E (1984) Glicentin-containing cells in intestinal metaplasia, adenoma and carcinoma of the stomach. Virchows Arch [Pathol Anat] 404:17–29

Ito H, Hata J, Yokozaki H, Tahara E (1986) Calcitonin in human gastric mucosa and carcinoma. J Cancer Res Clin Oncol (in press)

Mitschke H, Becker M (1973) Zur Cytopathologie der disseminierten endocrine Zellen des Magens bei Gastritis, Ulcus und Magencarcinom. Virchows Arch [Pathol Anat] 358:81–98

Nakamura S, Kino I, Baba S (1983) Ex vivo autoradiography of the human gastrointestinal tract: A new approach to cell kinetic studies of surgically removed tumor-bearing organs. Gann 74:116–121

Nemoto N, Kawaoi A, Shikata T (1982) Immunohistochemical demonstration of serotonin (5-HT)-containing cells in the human and rat intestines. Biomed Res 3:181–187

Resnick RH, Gray SJ (1961) Distribution of serotonin (5-Hydroxytryptamine) in the human gastrointestinal tract. Gastroenterology 41:119–121

Saik RP (1981) Serotonin as an inhibitor of gastrin. Scand J Gastroenterol 16:337–340

Singh I (1966) The distribution of cells of the enterochromaffin system in the gastrointestinal tract of human foetuses. Acta Anat 64:544–558

Solcia E, Polak JM, Pearse AGE, Forssmann WG, Larsson LI, Sundler F, Lechago J, Grimelius L, Fujita T, Creutzfeldt W, Gepts W, Falkmer S, Lefranc G, Heitz Ph, Hage E, Buchan AMJ, Bloom SR, Grossman MI (1978) Lausanne 1977 classification of gastroenteropancreatic endocrine cells. In: Bloom SR (ed) Gut hormones. New York, Raven Press, pp 40–48

Sternberger LA (1979) Immunochemistry. Wiley. New York, Chichester, Brisbane, Toronto

Tahara E, Ito H, Nakagami K, Shimamoto F, Yamamoto M, Sumii K (1982) Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme and HCG. Cancer 49:1904–1915

Tsutsumi Y, Nagura H, Watanabe K, Yanaihara N (1983) A novel subtyping of intestinal metaplasia of the stomach, with special reference to the histochemical characterizations of endocrine cells. Virchows Arch [Pathol Anat] 401:73–88

Yanaihara N (1980) Immunochemical application of synthetic peptides to studies on the prohormone-hormone system. Biomed Res 1:105–116

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