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A novel subtyping of intestinal metaplasia of the stomach, with special reference to the histochemical characterizations of endocrine cells

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Summary. Intestinal metaplasia of the stomach was grouped into 3 subtypes (A, B and C) according to the degree of pyloric gland involution which was judged from patterns of paradoxical Concanavalin A staining after Katsuyama and Spicer. The appearance of endocrine cells was investigated with immunohistochemical and silver methods. Type A metaplasia with slightly to moderately atrophic pyloric glands corresponded to the incomplete type in the previous classification, while Type C showing complete disappearance of pyloric glands corresponded to the complete type. Type B with severely atrophic pyloric glands was an intermediate. This subtyping reflects the cell kinetics in the intestinalized mucosa well. Regarding the endocrine cells, their total number varied in the order Type A > Type B > Type C. The selective populations of the endocrine cells including glicentin-containing cells, Grimelius-positive argyrophil cells without argentaffinity and intestinal-type enterochromaffin cells frequently formed hyperplastic foci in the intestinalized areas, where the other gut-type and proper gastric-type endocrine cells were scarcely noted. Immunoreactivity of glucagon or bovine pancreatic polypeptide were occasionally identified in a subpopulation of the glicentin-containing cells.

Key words: Immunoperoxidase method – Intestinal metaplasia of the stomach – Subtypes of intestinal metaplasia – Endocrine cells – Glicentin – Pancreatic polypeptide – Argyrophil cells – Enterochromaffin cells

Introduction

Intestinal metaplasia is known to be a unique phenomenon in the gastric mucosa as a result of long-standing inflammatory processes (Glass and Pitchumoni 1975). It has been subdivided into complete and incomplete

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types which are categorized by the appearance of Paneth cells and the expression of intestinal marker enzymes and sulfomucin (Abe et al. 1974; Kawachi et al. 1974; Matsukura et al. 1980). Cell kinetics in the metaplastic gastric mucosa have been autoradiographically analysed (Winawer and Lipkin 1969; Hattori and Fujita 1979; Hashimoto 1982). Argentaffin or enterochromaffin (EC) cells have long been noted to occur in intestinalized glands (Hamperl 1927) and the appearance of Grimelius-positive argyrophil cells and other gut-type endocrine cells containing somatostatin, glicentin, motilin and cholecystokinin (CCK) in the intestinalized gastric mucosa has been pointed out electron microscopically or histochemically (Hage 1976; Bordi et al. 1978; Bordi and Ravazzola 1979). Intra- and extraglandular hyperplasia of Grimelius-positive argyrophil cells in the mucosa with chronic atrophic gastritis has also been demonstrated (Solcia et al. 1979). Recently, we have reported on very conspicuous changes in the endocrine cells in this lesion: Intestinal metaplasia is characterized endocrinologically by 1) a dramatic decrease of gastric-type endocrine cells (gastrin-containing G cells and somatostatin-containing D cells), 2) a selective and preferential increase of three populations of endocrine cells including glicentin-containing cells, Grimelius-positive argyrophil cells and intestinal-type EC cells, and 3) the rarity or absence of other intestinal-type endocrine cells such as motilin-, neurotensin- and secretin-containing cells (Tsutsumi et al. 1983).

In this article, we propose a new classification of intestinal metaplasia according to the degree of pyloric gland involution. Changes of the endocrine cells in each subtype of the intestinal metaplasia were histochemically characterized in order to clarify the cell kinetics of the endocrine components in this lesion. The pathophysiological significance of the selective increase of glicentin-containing cells in the intestinalized gastric mucosa is also discussed.

Materials and methods

Patients. In all, 39 specimens of resected stomachs (17 for peptic ulcer, 20 for adenocarcinoma, 1 for malignant lymphoma and 1 for adenoma) were used. The age of the patients ranged from 22 to 78 (mean 53.6). Each stomach was fixed in 10–20% formalin for 1–7 days. One strip of the antropyloric area including a part of the duodenum where no neoplastic or ulcerative lesions were identified was embedded in paraffin and was cut at 4 μm thickness. Additional specimens of other areas such as the gastric angle and oxyntic mucosa were examined in some cases.

Mucin histochemistry. For the identification and characterization of the intestinal metaplasia, high iron diamine-Alcian blue (HID-AB) staining (Spicer 1965) was performed in addition to haematoxylin and eosin (H&E). To evaluate the degree of pyloric gland involution, paradoxical Concanavalin A staining which is highly specific for mucins of the pyloric glands, mucous neck cells and Brunner's glands (Katsuyama and Spicer 1978) was utilized; Briefly, diastase digested deparaffinized sections were oxidized in 0.5% periodic acid solution at room temperature (RT) for 60 min, reduced with 0.03% sodium borohydride in 1% dibasic sodium phosphate solution, pH 9.0 at RT for 3 min, reacted with 0.1% Concanavalin A (Sigma Chemical Co., MA, USA) in 0.01 M phosphate-buffered saline (PBS), pH 7.4 at RT for 15 min and then, after rinsing with PBS, reacted with 0.0005% horseradish peroxidase (HRP) (Sigma Chemical Co., MA, USA, type VI) at RT for 15 min. After thorough rinsing with PBS, the sections

were dipped at RT for 10 min in the diaminobenzidine (DAB) solution; 0.01 M Tris-HCl buffer, pH 7.6 containing 30 mg/dl of 3,3′-DAB tetrahydrochloride (Wako Pure Chemical Industries, Japan) and 0.01 M hydrogen peroxide. Nuclei were counterstained with 1% methylgreen solution, pH 4.0.

Silver methods. Each specimen was stained with Grimelius' silver for argyrophilia (Grimelius 1968) and with Fontana-Masson's silver for argentaffinity (Masson 1928). Counterstaining was performed with 0.03% lightgreen SF yellowish solution.

Antisera. The following antisera with known immunological specificities were used as primary antibodies for the immunohistochemistry. These include guinea pig anti-human little gastrin serum (GP-1304, Yanaihara), rabbit anti-cyclic somatostatin 1–14 serum (OAL-272, Japan Immunoresearch Laboratories Co.), rabbit anti-porcine glicentin C-terminal fragment 49–69 serum (R-4804, Yanaihara), rabbit anti-glucagon C-terminal fragment 19–29 serum (GC-5, Japan Immunoresearch Laboratories Co.), rabbit anti-bovine pancreatic polypeptide (PP) serum (615-R-110-146-17, Dr. RE Chance, Lilly Laboratories, USA), rabbit anti-porcine motilin serum (R-1104, Yanaihara), rabbit anti-neurotensin serum (R-3511, Yanaihara), rabbit anti-porcine secretin serum (R-801, Yanaihara), rabbit anti-porcine gastrin releasing peptide (GRP) serum (R-6903, Yanaihara), rabbit anti-porcine vasoactive intestinal polypeptide (VIP) serum (R-502, Yanaihara) and rabbit anti-substance P serum (R-2404, Yanaihara). Anti-gastrin serum is not cross-reactive to CCK (Yanaihara, C. et al. 1980). Anti-glicentin 49–69 serum recognizes an antigenic determinant in the C-terminal heptapeptide of the porcine glicentin sequence, which is linked to the C-terminus of the glucagon sequence and is common in the gut-type glucagon (enteroglucagon) and proglucagon (Yanaihara 1980). Anti-glucagon 19–29 serum reacts only to the pancreatic-type glucagon while glicentin and its equivalents are not detected (Imagawa et al. 1979).

For secondary antibodies, HRP-labeled goat IgG Fab fragment against rabbit IgG was prepared in our laboratory. HRP-labeled rabbit IgG against guinea pig IgG was purchased from Miles Laboratories, USA.

Immunohistochemistry. With the primary and secondary antisera listed above, the indirect immunoperoxidase method after Nakane (1975) was performed. Incubation with the antibodies and rinsing with PBS for each step were performed at RT for 30 min. The primary antisera were generally used at a 1:500–1:1,000 dilution. Endogenous peroxidase was inactivated by dipping deparaffinized sections in 0.5% periodic acid solution at RT for 10 min before the immunostaining and by adding 0.01 M sodium azide into the DAB solution. Nuclear counterstaining was performed with 1% methylgreen solution, pH 4.0.

In order to confirm the specificities of each immunostaining, the primary antisera preincubated with an excess amount of the corresponding antigens (finally 5 μg/ml) at 37° C for 1 h were used as negative controls. Cross-reaction of anti-gastrin serum to CCK was checked by the preincubation with synthetic human gastrin I (Calbiochem-Behring Co. USA), synthetic non-sulfated CCK octapeptide (Protein Research Foundation, Japan) and synthetic porcine CCK 1–27 (Yanaihara). Cross-reactions between anti-glicentin 49–69 serum and anti-glucagon 19–29 serum or anti-PP serum were also examined by the cross-immunoabsorption experiments with synthetic porcine glicentin 49–69 (Yanaihara), synthetic glucagon 22–29 (Penninsula Laboratories, USA) or purified bovine PP (Dr. RE Chance). For the detection of different antigens in single cells, "mirror sections" at 3 μm thickness were utilized (Osamura et al. 1980).

Results

Intestinal metaplasia was characterized by the appearance of goblet cells which were easily identified with HID-AB staining: Goblet cells usually contained sialomucin (colored blue) in the cytoplasm, but sulfomucin (colored black)-containing goblet cells were also occasionally noted. Of 39 antropyloric sections, 14 showed intestinal metaplasia occupying more than two thirds of the antropyloric mucosa (diffuse), 9 showed the lesion occupy-

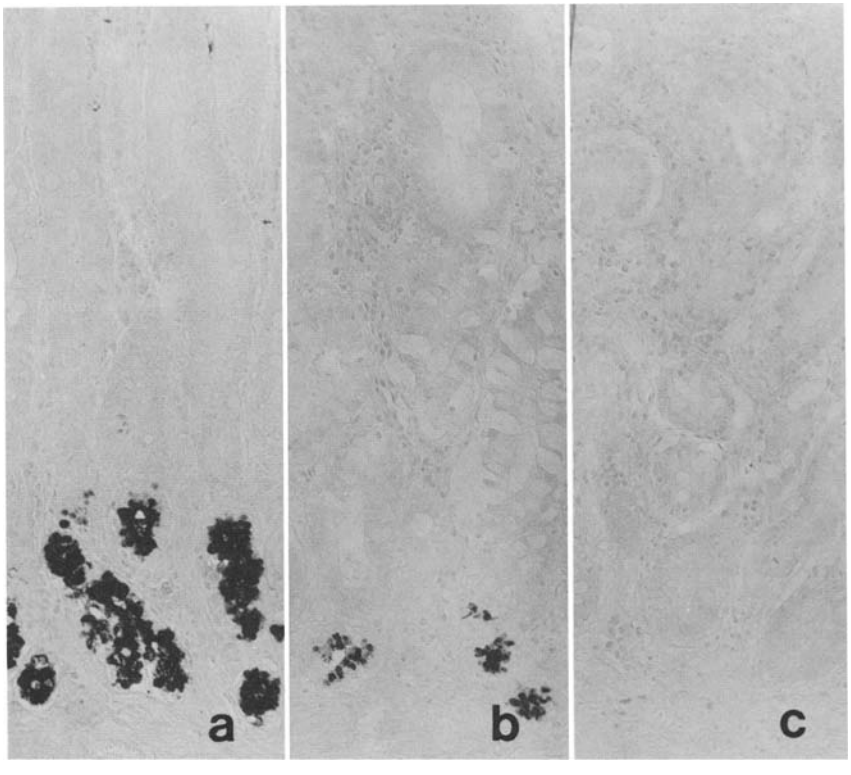

Fig. 1a–c. Subtypes A, B and C of intestinal metaplasia of the stomach. Paradoxical Concanavalin A staining for pyloric gland mucin. ×150. **a** Type A, **b** Type B, **c** Type C. Atrophic change in the pyloric glands is slight to moderate in Type A and severe in Type B. The pyloric glands are no longer seen in Type C

ing one third to two thirds (intermediate) and 8 showed the lesion occupying less than one third (focal). Eight sections revealed no metaplastic changes. In all, 369 metaplastic foci in the specimens were used for the semiquantitative evaluation of the endocrine populations. Each metaplastic focus comprized a group of metaplastic glands totally or partially replacing the area gastricae. Two hundred fourty-eight (67.2%) foci were from sections with diffuse metaplasia, 100 (27.1%) from sections with an intermediate range of metaplasia and 21 (5.7%) from sections with focal metaplasia.

Subtyping of intestinal metaplasia

In this study, intestinal metaplasia was divided into three subtypes according to the degree of involution of the pyloric glands. For this purpose, paradoxical Concanavalin A staining for pyloric gland mucin was utilized. Intestinal metaplasia with slightly to moderately atrophic pyloric glands was classified as Type A (Fig. 1a). In intestinal metaplasia Type B, markedly involuted pyloric gland cells were present only at the bottom of the intestinalized glands (Fig. 1b): The presence of the involuted pyloric glands in Type B

Table 1. Relationship between subtypes of intestinal metaplasia and Paneth cells

	The number of Paneth cells				Total
	−	(+)	+	++	
Type A	81 (86.2)	8 (8.5)	4 (4.3)	1 (1.1)	94 (100%)
Type B	112 (67.9)	10 (6.1)	32 (19.4)	11 (6.7)	165 (100%)
Type C	23 (20.1)	4 (3.6)	35 (31.8)	48 (43.6)	110 (100%)
Total	216 (58.5)	22 (6.0)	71 (19.2)	60 (16.3)	369 foci (100%)

++ = many; + = scattered; (+) = a few; − = no

Table 2. Relationship between subtypes of intestinal metaplasia and sulfomucin-containing goblet cells

	The number of sulfomucin-containing goblet cells				Total
	−	(+)	+	++	
Type A	28 (30.0)	10 (10.6)	30 (31.9)	26 (27.7)	94 (100%)
Type B	63 (38.2)	21 (12.7)	64 (38.8)	17 (10.3)	165 (100%)
Type C	69 (62.7)	13 (11.8)	24 (21.8)	4 (3.6)	110 (100%)
Total	160 (43.4)	44 (11.9)	118 (32.0)	47 (12.7)	369 foci (100%)

++ = many; + = scattered; (+) = a few; − = no

metaplasia was apt to be missed when observed with H&E staining alone. In Type C, no residual pyloric gland cells were identified: The mucosa was completely replaced by the intestinal-type glands (Fig. 1c). Type A and Type C metaplasia corresponded fairly well to the incomplete and complete types of the previous classification, respectively. Table 1 shows a relationship between each subtype of the intestinal metaplasia and the number of Paneth cells whose appearance accounts for a reliable characteristic of the complete type metaplasia. About 86% of Type A metaplasia lacked Paneth cells whereas 80% of Type C metaplasia possessed Paneth cells. In the most common Type B metaplasia, the data was intermediate between Types A and C: Paneth cells were absent in two thirds of Type B cases. Table 2 shows a relationship between the subtypes and sulfomucin (colored black with HID-AB staining)-positive goblet cells whose existence is known to be a marker for the incomplete type metaplasia. The number and fre-

Table 3. Relationship between the occurrence of each subtype and the extension range of intestinal metaplasia

Extent/Subtype	Type A	Type B	Type C	Total
Diffuse	59 (23.8)	116 (46.8)	73 (29.4)	248 (100%)
Intermediate	28 (28.0)	44 (44.0)	28 (28.0)	100 (100%)
Focal	7 (33.3)	5 (23.8)	9 (42.9)	21 (100%)
Total	94 (25.5)	165 (44.7)	119 (29.8)	369 foci (100%)

quency of sulfomucin-positive goblet cells tended to decrease in the order Type A > Type B > Type C. Table 3 shows the occurrence of each subtype in the gastric mucosa with diffuse, intermediate or focal intestinalization: Each subtype was observed at a relatively constant rate irrespective of the extent of intestinalization.

Histochemical observations of endocrine cells

Non-metaplastic antral mucosa. In non-metaplastic antral mucosa with variable degrees of inflammation, there were plentiful, and often hyperplastic, G cells (Fig. 2a) and fewer D cells, present mainly at the glandular neck region. A variable number of Fontana-Masson-positive gastric-type EC cells were distributed among the pyloric glands, where they lay at the "closed" position without a contact to the gland lumen. In addition, Grimelius-positive argyrophil cells other than the gastric-type EC cells were also scattered and their number tended to increase as the pyloric glands involuted. The immunohistochemistry for various hormones mentioned above failed to identify their hormonal contents. It is noteworthy that the non-metaplastic mucosa surrounded by or adjacent to the diffusely intestinalized areas frequently showed frank hyperplasia of G cells; and that in the mucosa with non-metaplastic atrophic gastritis, G cells, gastric-type EC cells and Grimelius-positive argyrophil cells without argentaffinity occasionally formed micronodules which consisted exclusively of several endocrine cells, located in the interstitium apart from the glands. The degree of pyloric gland involution in the non-metaplastic gastritic mucosa was often comparable to that in Type A metaplasia. Non-gastric-type endocrine cells described below were not demonstrated except for a few neurotensin-containing cells in some pyloric glands.

Intestinalized mucosa. Figure 2 shows a representative change of endocrine cells in metaplastic foci where glicentin-containing cells were preferentially

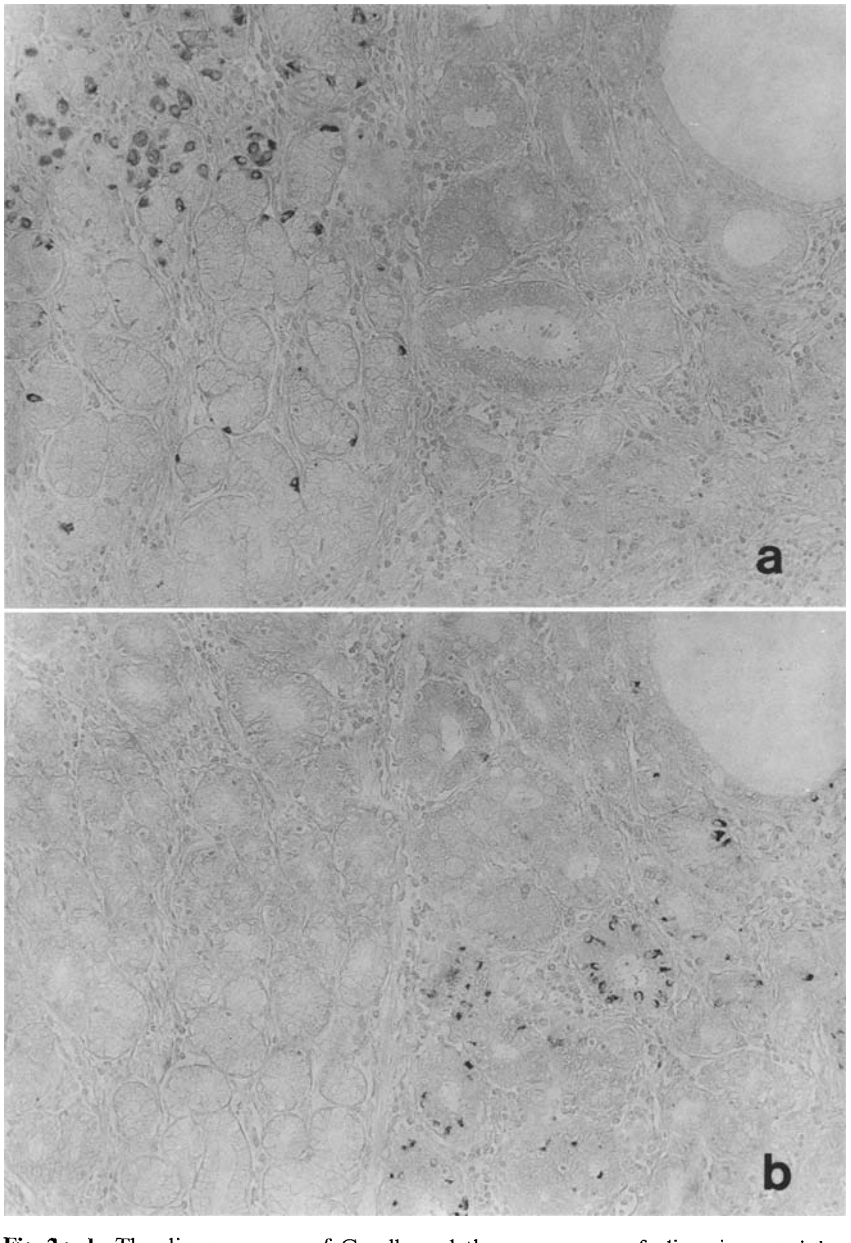

Fig. 2a, b. The disappearance of G cells and the appearance of glicentin-containing cells in the intestinalized antral mucosa (Type A). ×150. Indirect immunoperoxidase staining for gastrin **a** and glicentin **b**. G cells were distributed mainly at the glandular neck region of the non-metaplastic antral mucosa (*left half*), while the intestinalized area (*right half*) reveals numbers of glicentin-containing cells which lie in the deep portion of the intestinalized glands because of a downward shift of the generative cell zone. A drastic qualitative change of endocrine cells in the antrum induced by the intestinal metaplasia is evident

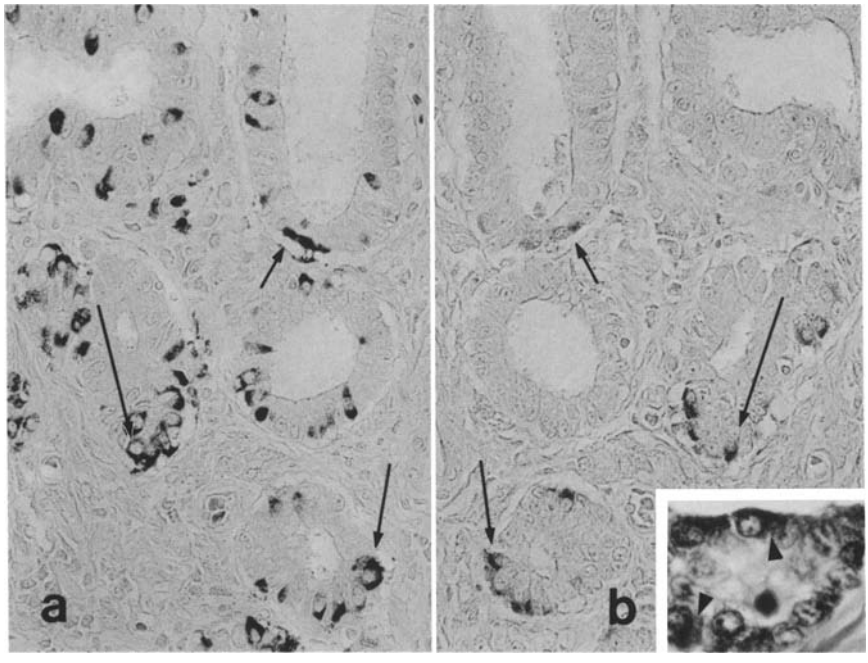

Fig. 3a, b. A pair of mirror sections of the intestinalized antral mucosa (Type A). ×300. **a** Grimelius' silver, **b** Fontana-Masson's silver. Fontana-Masson-positive EC cells are also argyrophilic (*arrows*), but argyrophil cells are far more numerous than the EC cells in this lesion. (*Inset:* Basal granules of the intestinal-type EC cells in the intestinalized glands show strong eosinophilia in H&E staining. ×600)

increased instead of G cells. The similar selective appearance in the intestinalized glands was observed for Grimelius-positive argyrophil cells and Fontana-Masson-positive intestinal-type EC cells (Figs. 3 and 4). These three endocrine components, especially the former two, fairly frequently showed frank hyperplasia in the intestinalized areas, where other kinds of the intestinal-type or pancreatic-type endocrine cells containing motilin, neurotensin, secretin, glucagon or PP were scarcely detected or absent. In contrast with the non-metaplastic mucosa, extraglandular micronodule formation by the hyperplastic endocrine cells was rare in the intestinalized mucosa. However, proper gastric-type endocrine cells such as G cells, D cells and gastric-type EC cells were drastically decreased or had disappeared from the intestinalized mucosa (Fig. 2a). Two pairs of mirror sections of Figs. 3 and 4 indicate that glicentin-containing cells, Grimelius-positive argyrophil cells without argentaffinity and intestinal-type EC cells comprize different endocrine cell populations. Most of the glicentin-containing cells failed to show argyrophilia except for a few cells in which glucagon immunoreactivity was also demonstrated. Argentaffin intestinal-type EC cells showed argyrophilia invariably, but the total number of them never exceeded that of the Grimelius-positive cells.

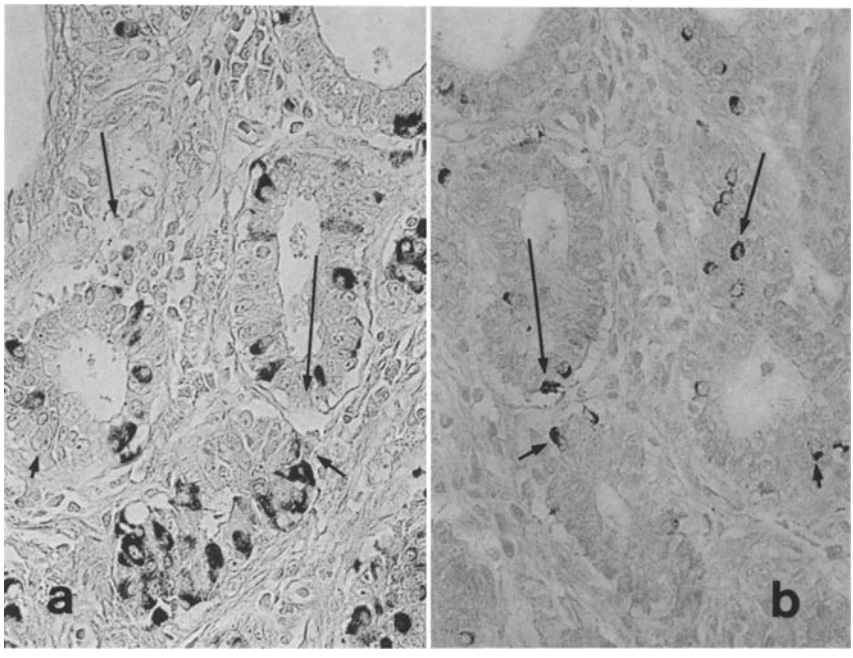

Fig. 4a, b. A pair of mirror sections of the intestinalized antral mucosa (Type A). ×300. **a** Grimelius' silver, **b** indirect immunoperoxidase staining for glicentin. Almost all of the glicentin-containing cells are not argyrophilic (*arrows*)

Regarding the intestinal-type EC cells, they were distinguished, by light microscopy, from gastric-type EC cells simply by their stainability with eosin dye and by their "open" position: Intestinal-type EC cells, whose slender cytoplasm showed direct contact to the gland lumen, exhibited basal cytoplasm with an eosinophilic granularity in H&E sections (Fig. 3, inset), whereas the gastric-type EC cells at the "closed" position without direct contact to the gland lumen did not.

Figures 5 and 6 present evidence of a simultaneous localization of glucagon or PP immunoreactivity within a subpopulation of the glicentin-containing cells. Such co-existence was fairly rare, but almost all immunoreactivity of glucagon or PP was demonstrated in the glicentin-containing cells.

Figure 7 illustrates a summary of the semiquantitative analysis of each endocrine component in the subtypes (A, B and C) of the intestinal metaplasia. In all, 369 metaplastic foci were examined (Type A, 94 foci; Type B, 165 foci; and Type C, 110 foci). The number and appearance rate of each endocrine component in the subtypes of the intestinal metaplasia generally varied in the order Type A > Type B > Type C. Namely, in Type A metaplasia, a hyperplastic increase of two out of the three major components was most frequent, and the occurences of gastrin, somatostatin, motilin, PP and glucagon were observed at a higher rate. (Hyperplasia of glicentin-containing cells was most frequent in Type B.) It should be emphasized

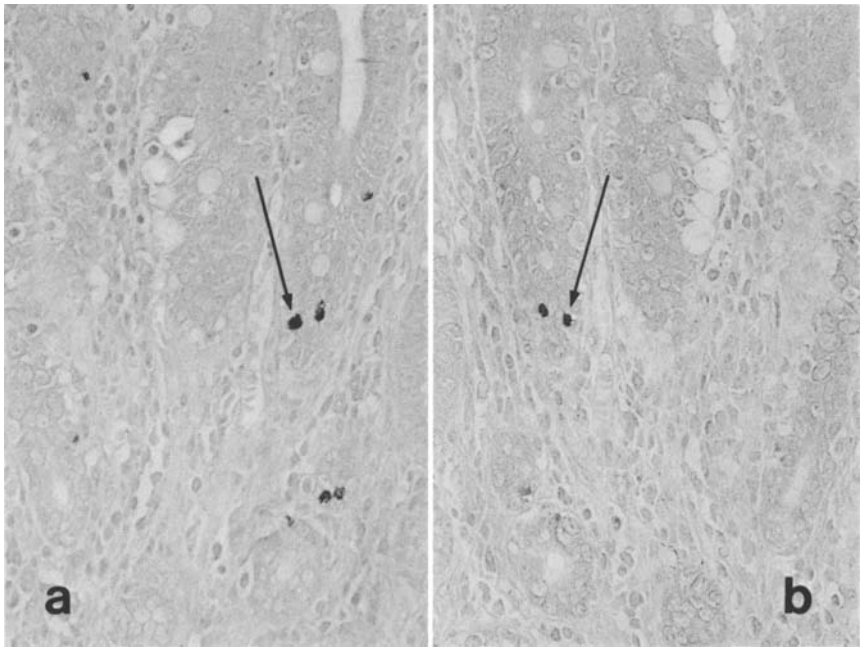

Fig. 5a, b. A pair of mirror sections of the intestinalized antral mucosa (Type A). ×300. Indirect immunoperoxidase staining for glicentin **a** and glucagon **b**. A subpopulation of the glicentin-containing cells also show glucagon immunoreactivity (*arrows*)

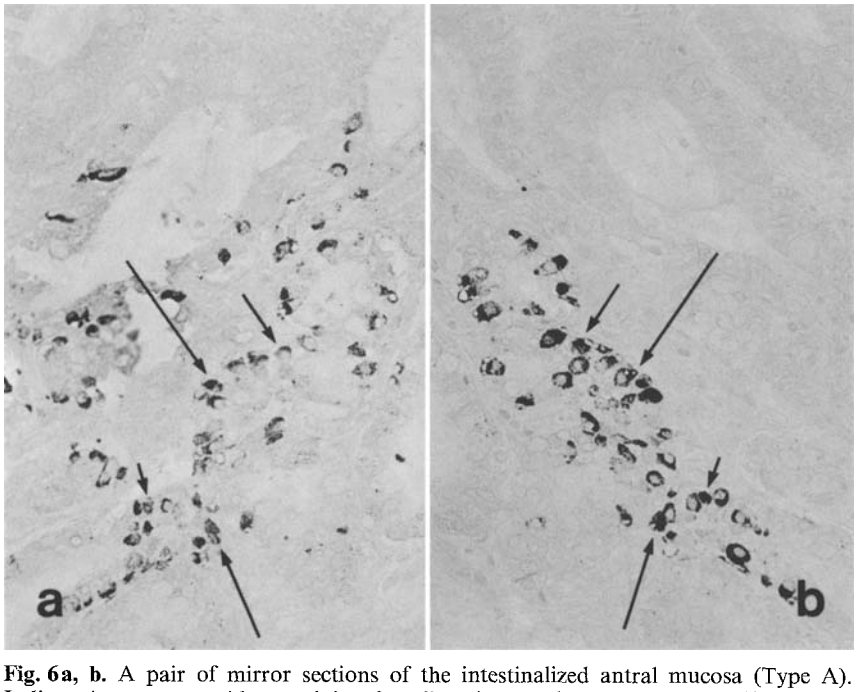

Fig. 6a, b. A pair of mirror sections of the intestinalized antral mucosa (Type A). ×300. Indirect immunoperoxidase staining for glicentin **a** and *PP* **b**. Over a half of the glicentin-containing cells in this area reveal PP immunoreactivity simultaneously

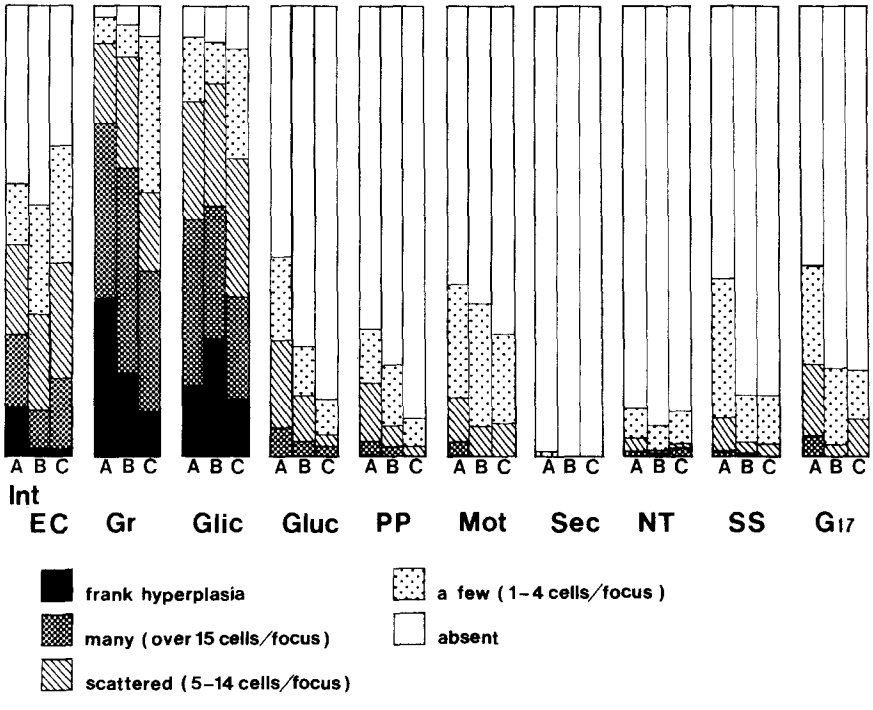

Fig. 7. Diagrammatic representation of the relative volumes of endocrine components in each subtype (A, B and C) of the intestinal metaplasia. [*Abbreviations: Int EC* = intestinal-type enterochromaffin cells, *Gr* = Grimelius-positive argyrophil cells, *Glic* = glicentin cells, *Gluc* = glucagon cells, *PP* = bovine pancreatic polypeptide cells, *Mot* = motilin cells, *Sec* = secretin cells, *NT* = neurotensin cells, *SS* = somatostatin cells and *G17* = gastrin cells]

that a pronounced increase of the total number of endocrine cells also characterized the intestinal metaplasia. Endocrine cell hyperplasia was demonstrated in 169 of 369 metaplastic foci (45.8%); in 58 of 94 foci (61.7%) of Type A metaplasia, in 83 of 165 (50.3%) of Type B and in 28 of 110 (25.5%) of Type C. The number of metaplastic foci with a few endocrine cells was only 7 (1.9%); none in Type A (0%), 2 in Type B (1.2%) and 5 in Type C (4.5%).

Endocrine cells containing GRP, VIP or substance P were not detected at all in the gastric mucosa: Only nerve fibers were positive for these neuropeptides.

The above-mentioned findings were not confined in the antral mucosa: Samples from the gastric angle and oxyntic mucosa also revealed a comparable phenomenon except for the absence of G cells in oxyntic mucosa. (In the oxyntic mucosa, the intestinalization was preceded by the so-called pseudo-pyloric gland metaplasia.)

Duodenal mucosa. The duodenal mucosa in each section commonly revealed scattered or many gastrin-, somatostatin- or motilin-containing cells as well as intestinal-type EC cells and Grimelius-positive argyrophil cells without

argentaffinity. The number of secretin-containing cells was usually small. In some cases, a few neurotensin- or PP-containing cells were also present. The detection of glicentin-containing cells in the duodenal mucosa was very exceptional. Cells containing glucagon, GRP, VIP or substance P were not found. It is worthy of note that the normal duodenal mucosa scarcely showed such a degree of the endocrine cell hyperplasia as the metaplastic gastric mucosa frequently did.

Specificities of the immunostaining. Immunoabsorption tests with an excess amount of the corresponding synthetic or purified antigens proved the specificities of the antisera used. Cross-reaction of anti-gastrin antiserum to CCK-octapeptide or CCK 1–27 was similarly ruled out. Cross-immunoabsorption experiments with porcine glicentin 49–69, glucagon 22–29 and bovine PP also confirmed the specificities of the respective immunostainings for glicentin, glucagon and PP.

Discussion

Intestinal metaplasia of the stomach used to be subdivided into two types, complete and incomplete (Abe et al. 1974; Kawachi et al. 1974; Matsukura et al. 1980). The complete type is characterized by the total expression of the intestinal marker enzymes, the absence of sulfomucin-containing goblet cells and the presence of Paneth cells as in the mucosa of normal small intestine (Matsukura et al. 1980). In the incomplete type, the expression of the intestinal marker enzymes is partial, sulfomucin is identified in goblet cells and Paneth cells are absent, and it has been suggested that the incomplete type metaplasia has a closer relationship to gastric cancer than the complete type (Matsukura et al. 1980).

Meanwhile, cell kinetics in the intestinalized gastric mucosa has also been investigated with autoradiography (Winawer and Lipkin 1969; Hattori and Fujita 1979; Hashimoto 1982). Hashimoto (1982) has shown that the generative cell zone in the intestinalized glands with a diffuse alkaline phosphatase activity (corresponding to the complete type) is located only at the bottom of the glands, while the generative cell zone in the intestinalized glands with a focal or no alkaline phosphatase activity (corresponding to the incomplete type) is widely distributed in the lower half of the glands. The same author has also pointed out that brush border formation is abortive in the intestinalized glands with a wide generative cell zone (Hashimoto 1982). Hattori and Fujita (1979) have reported that in the totally intestinalized mucosa in which all pyloric gland cells become lost, the generative cell zone is located at the bottom of the glands as it is in the normal intestine, and that in partially intestinalized mucosa which consists of the upper intestinal-type epithelial cells and the lower involuting pyloric gland cells, the generative cell zone lies at the glandular neck region and gradually shifts downwards from the isthmus to the bottom of the glands in proportion to the degree of involution of the pyloric glands. It can be said that the intestinalized mucosa involving the remaining pyloric glands would stay in an "unstable" or transitional status from the view of the cell kinetics (Fujita; personal communication).

Our present classification (Types A, B and C) of the intestinal metaplasia is based on the degree of pyloric gland involution, which could be easily and objectively judged with paradoxical Concanavalin A staining after Katsuyama and Spicer (1978). This method specifically stains mucins of the pyloric glands in the gastric antrum. Since the position of the generative cell zone in the intestinalized gastric mucosa is dependent on the degree of pyloric gland involution (Hattori and Fujita 1979), this subtyping would closely reflect the cell kinetics in the intestinalized glands. The appearance of Paneth cells and sulfomucin-containing goblet cells was fairly well correlated to the degree of pyloric gland involution (Tables 1 and 2). Thus, Type A metaplasia with fairly preserved pyloric glands corresponds to the incomplete type of the previous classification, while Type C without pyloric gland cells corresponds to the complete type. Type B would represent an intermediate form. In fact, transitional forms between the complete and incomplete types of intestinal metaplasia have been frequently recognized (Hashimoto 1982).

The extension of intestinal metaplasia in the gastric mucosa is known to progress slowly with age from the antrum to the body (Sugano et al. 1982). Intestinalized mucosa with remaining pyloric glands (Types A and B) was very frequently seen irrespective of the extent of intestinalization (Table 3). The pyloric gland cell is a mature cell with a limited life span which is calculated about 15–30 days in hamsters (Hattori and Fujita 1979). It is logical, therefore, that the pyloric gland cells in foci with Types A and B metaplasia should still be recruited from the generative cell zone. The cellular renewal in the mucosa with Types A and B metaplasia could be bidirectional: The generative cells would give rise to the intestinal-type epithelial cells upwards and to the pyloric gland cells downwards. This possible bidirectional cell kinetics can be taken as a partial modification of that in the normal antral mucosa (Hattori et al. 1977; Hattori and Fujita 1979). On the other hand, in Type C metaplasia, the mode of cellular renewal would be turned to that seen in the intestinal mucosa. In order to understand the intestinal metaplasia dynamically as a pathological or precancerous condition of the stomach, the changes in this cellular flow seem to be more essential than the secondary phenotypic expressions such as the appearance of Paneth cells, sulfomucin-containing goblet cells and alkaline phosphatase activity. The present subtyping of intestinal metaplasia would certainly be of benefit in this context.

Regarding the endocrine cells in the intestinalized glands, argentaffin or EC cells have long been noted (Hamperl 1927). However, few reports on the appearance of other kinds of endocrine cells in the intestinalized gastric mucosa have been published (Hage 1976; Bordi et al. 1978; Bordi and Ravazzola 1979). We have recently reported on very conspicuous changes of the endocrine cells in the intestinal metaplasia: Most characteristic is a pronounced increase of the total number of the endocrine cells, mainly consisting of three populations; glicentin-containing cells, Grimelius-positive argyrophil cells without argentaffinity and fewer intestinal-type EC cells (Tsutsumi et al. 1983). A drastic decrease of the gastric-type endocrine cells also characterizes this lesion (Tsutsumi et al. 1983). The present report fur-

ther demonstrates that the number and variety of the endocrine cells are largest in Type A metaplasia and smallest in Type C.

The preferential increase in certain populations of endocrine cells, especially in intestinalized mucosa with "unstable" cell kinetics, may exclude the possibility that the intestinalization is a simple aberrant expression of the intestinal-type epithelium in the gastric mucosa. In fact, such a degree of hyperplasia of the endocrine cells as is frequently observed in intestinal metaplasia was scarcely seen in the normal duodenal mucosa. Hence, the pathophysiological significance of the selectively increased endocrine cell populations in the intestinalized mucosa should be evaluated.

Intestinal-type EC cells with an "open" position might function through serotonin secretion (Facer et al. 1979) as a regulator of the local circulation in response to direct stimuli from the gland lumen. Biological functions of glicentin, an equivalent of enteroglucagon or proglucagon (Yanaihara 1980), have recently been elucidated. Bataille et al. (1981) have isolated from the porcine gut a peptide named "oxyntomodulin" which suppresses gastric acid secretion. This peptide is identical to the C-terminal fragment of glicentin, against which the anti-glicentin serum R-4804 used in this study is directed (Yanaihara 1980). Bloom and his collegues have reported a trophic action of enteroglucagon or glicentin on the intestinal epithelial cells as "a growth hormone to the gut" (Bloom and Polak 1981; Jacobs et al. 1981). Thus, glicentin, which is normally distributed in the lower intestinal tract, especially in the colon (Solcia et al. 1979), may have an important role for the maintenance of the intestinalized glands in the stomach: Hyperacidity may be harmful to the intestinal-type epithelial cells and their heterotopic presence in the gastric mucosa should require a high local level of the trophic hormone glicentin.

The simultaneous localization of pancreatic-type glucagon and PP in a subpopulation of the glicentin-containing cells in the intestinalized mucosa is identical to the finding in the normal human colonic mucosa (Fiocca et al. 1980; Lehy et al. 1981) and in the human fetal oxyntic mucosa (Tsutsumi, unpublished data). Although the functional importance of the coexistence remains unknown, it might be supposed that roles or functions of the glicentin-containing cells in the intestinalized mucosa are similar to those in the normal colonic or fetal oxyntic mucosa.

As to Grimelius-positive argyrophil cells without argentaffinity, their hormonal contents could not be determined in this study, and whether they comprize a single cell population is also unclear: Several kinds of endocrine cells in the gastrointestinal tract are known to be argyrophilic with Grimelius' silver, and both the intraglandular hyperplastic change and extraglandular micronodule formation by the argyrophil cells have been reported in the mucosa with atrophic gastritis (Bordi et al. 1978; Solcia et al. 1979). Identity or non-identity between the hyperplastic Grimelius-positive cells with extraglandular micronodule formation in the mucosa with non-metaplastic atrophic gastritis and those without micronodule formation in the intestinalized mucosa should be investigated with the aid of electron microscopy.

Attention should be paid to the fact that the endocrine components

qualitatively change from the gastric-type to the intestinal-type in a very abrupt fashion once the intestinal-type epithelial cells begin to appear at the generative cell zone. In the non-metaplastic mucosa with atrophic gastritis where a number of the gastric-type endocrine cells are distributed, the degrees of pyloric gland involution are often comparable to those in Type A metaplasia. In both conditions, the cell kinetics would be similarly bidirectional. It has been widely approved that the ontogenetic origin of the gastrointestinal endocrine cells is endodermal and they are continuously renewed from the generative cell zone (Hattori et al. 1977; Sidhu 1979). Hence, the switching for the gene expression of the hormones would occur synchronously with the appearance of the intestinal-type epithelial cells, especially goblet cells, at the partially altered generative cell zone. A similar synchronized phenomenon between the endocrine cells and goblet cells has been reported in the gallbladder mucosa: The normal gallbladder mucosa is almost devoid of both endocrine and goblet cells, and the appearance of the endocrine cells is mostly confined in the intestinalized areas (Laitio 1975). The precise mechanisms of the synchronized switching of the hormonal expression should be further investigated at the molecular biology level.

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