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Epithelial Phenotypic Expression of Human Foetal Gastrointestinal Mucosa: An Immunohistochemical Analysis

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ABSTRACT

Epithelial phenotypic expression in the gastrointestinal mucosa of 20 human foetuses from the 4th to 10th month of gestation was examined immunohistochemically for glycoproteins, enzymes, peptide hormones and serotonin. Goblet cells with neutral and sialomucin and brush-bordered absorptive cells were scattered in the cardia and antrum of the foetal stomach. Lysozyme was present in Paneth cells of the small intestine but not in the colon throughout the foetal period, whereas in the stomach it was seen in the surface epithelium and gastric glands in the second trimester and then became confined to pyloric and cardiac glands with the development of foetus. Secretory component was not detected in the foetal gastric mucosa but expressed in apical and basolateral cell membranes of the intestinal epithelium from the 10th gestational month. Using monoclonal antibody to carcinoembryonic antigen (CEA), only apical and lateral cell membranes of colonic epithelium from the 6th to 8th month of gestation were immunoreactive for CEA, but the stomach and small intestine had no CEA. Alpha-1-antitrypsin and alpha-1-antichymotrypsin were observed scattered in the crypts of the small intestine but were not detected in the stomach and colon. Gastrin containing cells were less in number than somatostatin containing cells in the stomach but dramatically increased from the 8th month of gestation. Serotonin containing cells showed the closed type in the stomach, the open type in the small intestine and both in the colon of the foetus. Glicentin containing cells were present in the fundic mucosa from the 5th to the last gestational month. Calcitonin was not detected in the foetal gut mucosa. These results suggest that the expression of these tissue markers in intestinal metaplasia and adenocarcinoma of the stomach cannot be explained merely by the reversion to the foetal gastric phenotype.

Gastric adenocarcinoma is well known to show not only wide variation in histological appearance but also immunoreactivities for tissue markers such as lysozyme[59,60], secretory component[24,46,56], protease inhibitors[33,61] and carcinoembryonic antigen[17,46,58]. Moreover, the occurrence of polypeptide hormones in gastric carcinoma has been described by many investigators[4,21,57]. Tahara et al have also confirmed immunohistochemically that endocrine cells producing several polypeptide hormones are found in 20 to 30% of gastric adenocarcinoma cases[58,60]. These morphological and functional multiplicities of human gastric adenocarcinomas are frequently thought to account for the reversion of tumour cells to foetal expression.

Since the report of Ascoli (1901), intestinal type cells including goblet cells and brush-

bordered epithelium have been observed in the foetal gastric antrum and cardia[3,8,49]. The intestinal heterotopia in the foetal stomach are considered to be closely related to the development of intestinal metaplasia in the adult gastric mucosa[5]. Deren (1971) suggested that intestinal metaplasia might be associated with a return to the above mentioned foetal primitive cells[10].

However, ontogenic expressions of glycoproteins, enzymes and polypeptide hormones in the foetal digestive tract mucosa have not yet been well established. The purpose of this immunohistochemical study on the foetal gastrointestinal tract mucosa is to elucidate the expression of several glycoproteins, enzymes and polypeptide hormones and serotonin in the developing human gut and to compare the results obtained with those of adenocarcinoma or intestinal metaplasia of the stomach.

MATERIALS AND METHODS

Studies were made on 20 human foetuses from the 4th to 10th month of gestation extracted from the files of the Department of Geneticopathology, Research Institute for Nuclear Biology and Medicine, Hiroshima University (Table 1). They were obtained by therapeutic abortion and at autopsy within 3 hr after death. No external or internal anomaly was observed in these foetuses. One or two representative 10% formalin fixed paraffin embedded blocks of the gastrointestinal tract were selected from each case for light microscopy and immunohistochemistry. Adjacent or serial sections cut 4 μm in thickness from these blocks were stained with hematoxylin and eosin, periodic acid Schiff (PAS), alcian blue (AB), high iron diamine (HID), Grimelius' silver nitrate technique for argyrophil reaction and Fontana-Masson's silver impregnation method for argentaffinity. Epithelial mucosubstances were classified after Spicer et al[53].

Antisera and Monoclonal Antibodies

Rabbit antisera to lysozyme, secretory component, alpha-1-antitrypsin, alpha-1-antichymotrypsin and calcitonin were purchased from DAKO Immunoglobulins (Copenhagen, Denmark) and employed at 1:800 dilution. Preparation and characterization of rabbit anti-gastrin antiserum have been described previously[58]. Rabbit anti-somatostatin serum (JG-1) and glucagon C-terminal specific antiserum (OAL123) were obtained from Japan Immunoresearch Laboratories Co. Ltd. (Takasaki, Japan) and lyophilized prepa-

Table 1. Foetuses used in this study

Case No.	Gestational age[a] (months)	Sex	C-R length[b] (cm)	C-H length[c] (cm)
1.	4	M[d]	9.0	14.5
2.	5	F[e]	15.0	23.0
3.	5	M	12.5	17.5
4.	5	F	13.0	20.0
5.	5	F	19.0	30.0
6.	6	M	16.5	26.5
7.	6	F	20.0	38.0
8.	6	F	21.0	33.0
9.	6	M	17.0	28.0
10.	7	F	23.0	36.5
11.	7	M	22.5	36.0
12.	8	F	24.0	39.5
13.	8	F	27.0	45.0
14.	8	M	26.0	40.5
15.	9	M	29.0	48.0
16.	10	M	34.0	55.5
17.	10	F	34.0	54.0
18.	10	F	35.0	54.0
19.	10	M	38.0	60.0
20.	10	M	35.0	60.0

[a]estimated from maternal obstetrical history, [b]C-R length; Crown-rump length, [c]C-H length; Crown-heel length, [d]M; male, [e]F; female

rations were diluted 1:800. Rabbit anti-glicentin antiserum (R4804) and anti-big gastrin antiserum (R2703) were kindly provided by Prof. N. Yanaihara, Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy, Shizuoka, Japan, and employed at 1:1000 dilution[68]. Anti-motilin antiserum (rabbit) was purchased from Cambridge Research Biochemicals (USA) and were used at 1:2000 dilution. Rat monoclonal anti-serotonin antibody was obtained from sera-lab (England) and used at 1:500 dilution. Anti-carcinoembryonic antigen (CEA) mouse monoclonal antibody CEM010 was provided by Mochida Pharmaceutical Co. (Tokyo, Japan) and employed at 1:800 dilution. Preparation and characterization of CEA-monoclonal antibody CEM010 have been described previously and it does not cross react with non-specific cross reacting antigen (NCA)[22,67]. Biotinylated anti-rabbit IgG, anti-mouse IgG, anti-rat IgG, avidin DH and biotinylated horse radish peroxidase were purchased from Vector Laboratories Inc. (Burlingame, USA), and used according to the supplier's recommendations.

Immunohistochemistry

Avidin-biotin-peroxidase complex (ABC) method after Hsu et al[23] was applied for immunostaining of each antigen. Incubation with antibodies or rinsing in 0.01 M PBS, pH 7.2 in each step was performed for at least 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. Following incubation in ABC, a coloured reaction product was formed using 0.003% 3,3'-diaminobenzidine-tetrahydrochloride (DAB; Sigma, Grade II) and 0.001% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6) for 5 to 10 min. The reaction was stopped in water and the sections were counterstained with 3% methylgreen.

The specificity of each primary antiserum and each step of reaction was determined according to a modification of the method of Sternberger[55] and reported elsewhere[25,27,28,56,58,59]. For the negative controls, nonimmune rabbit, mouse or rat IgG at 1:100 dilution were utilized in place of primary antibodies. Appropriate positive control slides were also stained at the same time.

Quantification

The distribution density of gastrin, somatostatin and glicentin containing cells in developing gastric mucosa was estimated by counting the number of immunoreactive cells and all nucleated epithelial cells in serial sections stained by hematoxylin and eosin. Then the quotient (number of immunoreactive cells / number of all nucleated cells) was calculated.

RESULTS

1. Histological changes in the gastrointestinal mucosa during intrauterine life

Histological changes of the foetal gastrointestinal mucosa were almost the same as reported previously[19,49–51], A few goblet cells and brush-bordered epithelial cells were sometimes observed in the antrum and cardia of the foetal stomach (Fig. 1a). The goblet cells in the stomach contained PAS-positive neutral mucin and AB positive sialomucin but no HID-positive sulfomucin (Fig. 1b). Segi's caps[31,50], the huge aggregations of endocrine cells, were observed at the top of villi in the small intestine from the 5th to 7th month of gestation. Goblet cells had neutral and sialomucin in the small intestine and sulfomucin and sialomucin in the large intestine.

2. Lysozyme (Ly)

The expression of Ly immunoreactivity in the human foetal gastrointestinal mucosa was different among the stomach, small intestine and large intestine. From the 4th to 6th month of gestation, the luminal surface and lateral side of the surface epithelium were positively stained by Ly at all areas of the gastric mucosa (Fig. 2a). Deep portions of the pyloric and cardiac glands were also immunoreactive for Ly (Fig. 2b). From the 7th month of gestation, the immunoreactive Ly in the surface epithelium began to disappear, and then at the 10th month it was localized to the pyloric and cardiac glands (Fig. 2c and d). Paneth cells had Ly immunoreactivities at every portion of the small intestine including Segi's caps (Fig. 2e and f). The mucosa of the large intestine showed no positive reaction for Ly during the foetal period.

3. Secretory component (SC)

SC immunoreactivity was not expressed in the gastric mucosa of all the foetal periods.

In the small and large intestines, it was not detected from the 4th to 9th month of gestation, but began to be observed at apical and

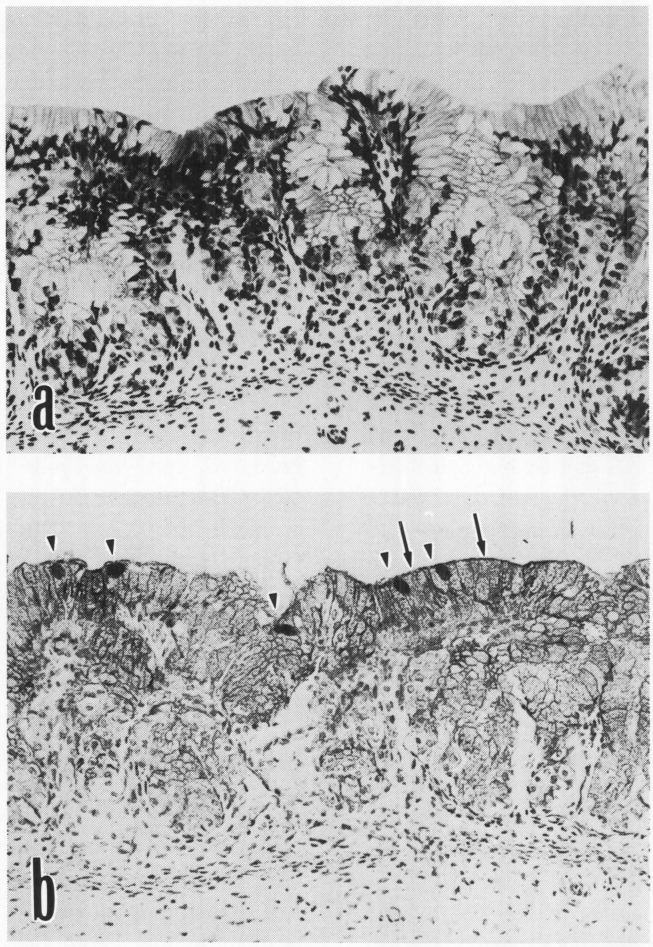

Fig. 1. Serial section prepared from antral gastric mucosa from a foetus at the 5th gestational month. (a) Hematoxylin and eosin (×150). (b) Some goblet cells are scattered in the surface epithelium (arrow heads) and brush-bordered epithelium exist around them (arrows). Periodic acid Schiff reaction (×150).

basolateral membranes of the epithelial cells at the deep portion of the crypt from the 10th month of gestation (Fig. 3).

4. Carcinoembryonic antigen (CEA)

Immunoreactive CEA could not be detected in the stomach and small intestine throughout the foetal period using monoclonal anti-CEA antibody CEM010 (Fig. 4a,b).

In the large intestine, apical and lateral membranes of the absorptive cells from the 6th to 8th month of gestation were immunoreactive to CEM010 monoclonal antibody (Fig. 4c). The immunoreaction disappeared from the 9th to the last gestational month (Fig. 4d).

5. Protease inhibitors

Alpha-1-antitrypsin (AAT) and alpha-1-antichymotrypsin (ACT) immunoreactivities were negative in the mucosa of the foetal stomach and large intestine at all stages of intrauterine life.

In the small intestine, AAT immunoreactivity was observed from the 6th month of gestation. They were seen in the basal epithelial cells of developing intestinal crypts, which were not identical with Paneth cells (Fig. 5a). At the 5th months of gestation, ACT immunoreactivity was observed in some epithelial cells of the intestinal crypts (Fig. 5b).

6. Endocrine cells

1) Little gastrin (G-17) and big gastrin (G-34)

In the stomach, G-17 immunoreactivity was observed in open type endocrine cells at the deep portion of the antral gastric gland from the

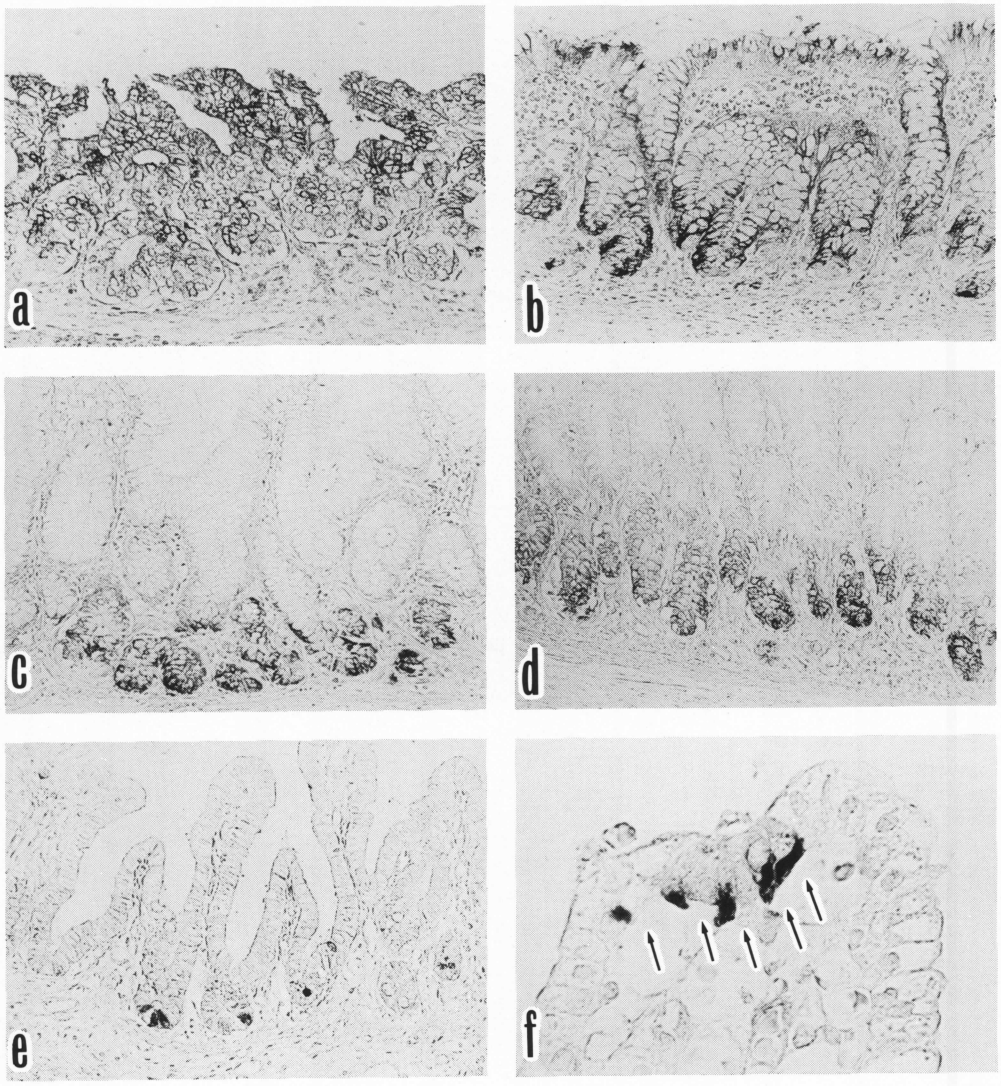

Fig. 2. Lysozyme immunostaining of foetal gastrointestinal tract. (a) Membranes of whole epithelial cells are immunoreactive for lysozyme. Gastric fundus at the 5th month of gestation (×178). (b) Cell membranes of surface mucous cells and cytoplasm of pyloric gland epithelia show positive immunoreaction for lysozyme. Immunostaining is more intense in the latter than the former. Pyloric region at the 6th gestational month (×140). (c) Immunoreactive lysozyme is localized to pyloric glands (×104) and (d) cardiac glands (×104) in the stomach at the 10th gestational month. (e) Lysozyme immunoreactivity is restricted to Paneth cells in the small intestinal mucosa (×104) and some cells of Segi's caps (f(arrows); ×274). 6th month of gestation.

4th month of gestation (Fig. 6a). G-34 immunoreactivity was also seen in the deep portion of the antral gastric glands but less in number than G-17 immunoreactivity and appeared firstly at the 5th gestational month. The number of G-17 or G-34 immunoreactive cells gradually increased and became localized in the middle portion of the pyloric gland with the development of the foetus (Fig. 7). In the small intestine, G-17 and G-34 immunoreactivities were observed in open type endocrine cells localized in the intestinal crypt, middle portion of the villi and Segi's caps from the 5th month of gestation (Fig. 6b,c). In the large intestine, G-17 and G-34 immunoreactivities were not observed throughout the foetal period.

2) Pancreatic glucagon and glicentin

Open type and closed type endocrine cells of

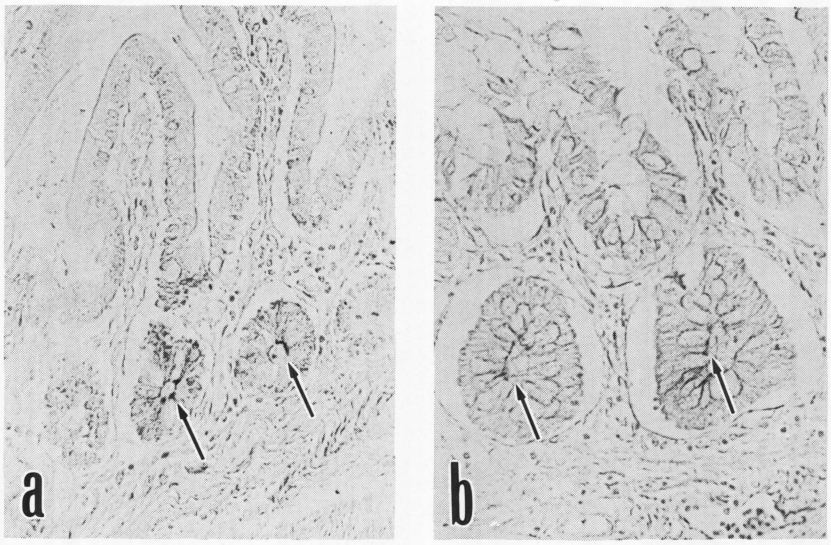

Fig. 3. (a) Secretory component is expressed at the 10th month of gestation in the apical and lateral membrane (arrows) of the crypt cells of the foetal small intestine (×215) and colon (b; ×215). Immunostaining with anti-secretory component serum.

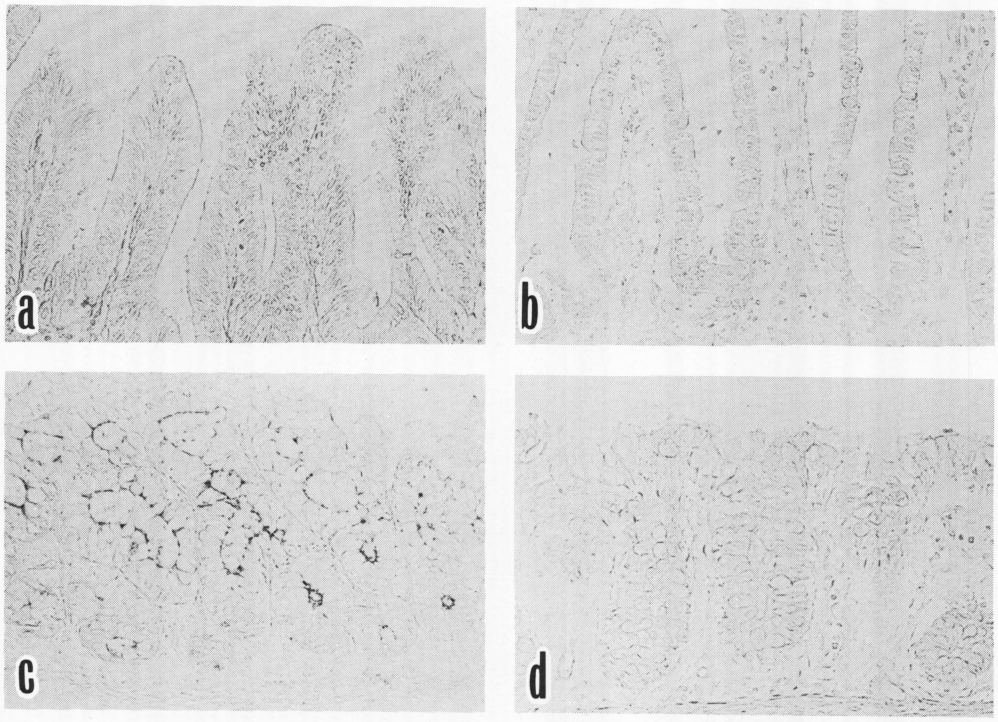

Fig. 4. CEA immunoreactivity in human foetal gastrointestinal mucosa. Immunoreactive CEA is not observed in the gastric mucosa (a; ×126) and small intestine (b; ×126) throughout the intrauterine period. (c) Immunoreactive CEA is detected at apical and lateral membranes of colonic epithelium. 6th gestational month (×126). (d) CEA immunoreactivity disappears from the colonic epithelium at the 10th month of gestation (×126). Immnostaining with anti-CEA monoclonal CEM010 antibody.

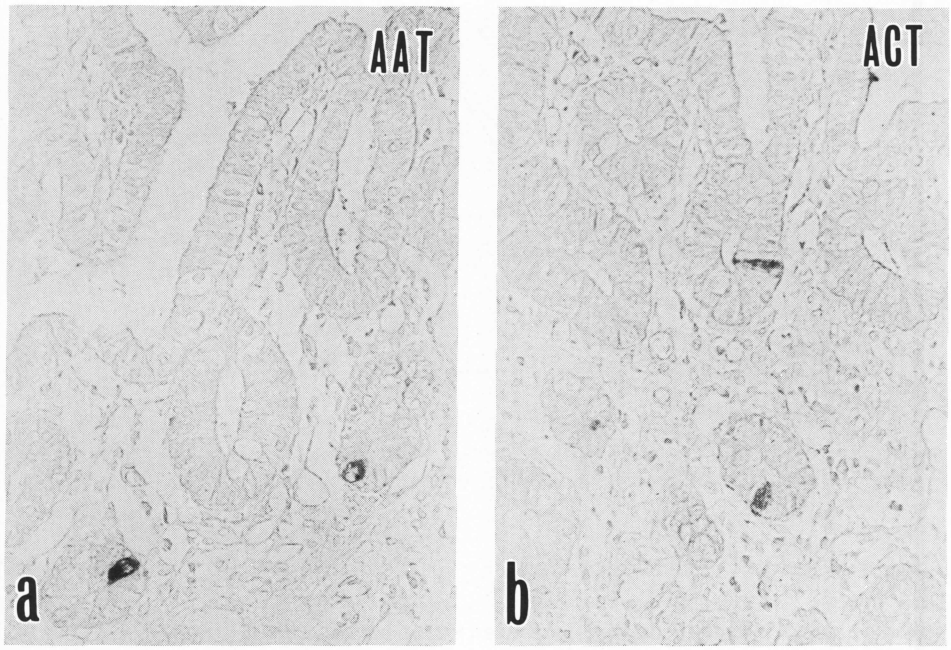

Fig. 5. (a) Alpha-1-antitrypsin immunoreactivity in the foetal small intestine is seen in the crypt cells. 10th month of gestation (×250). (b) Alpha-1-antichymotrypsin is also observed in the crypt cells. Small intestine from a foetus at the 10th gestational month (×250).

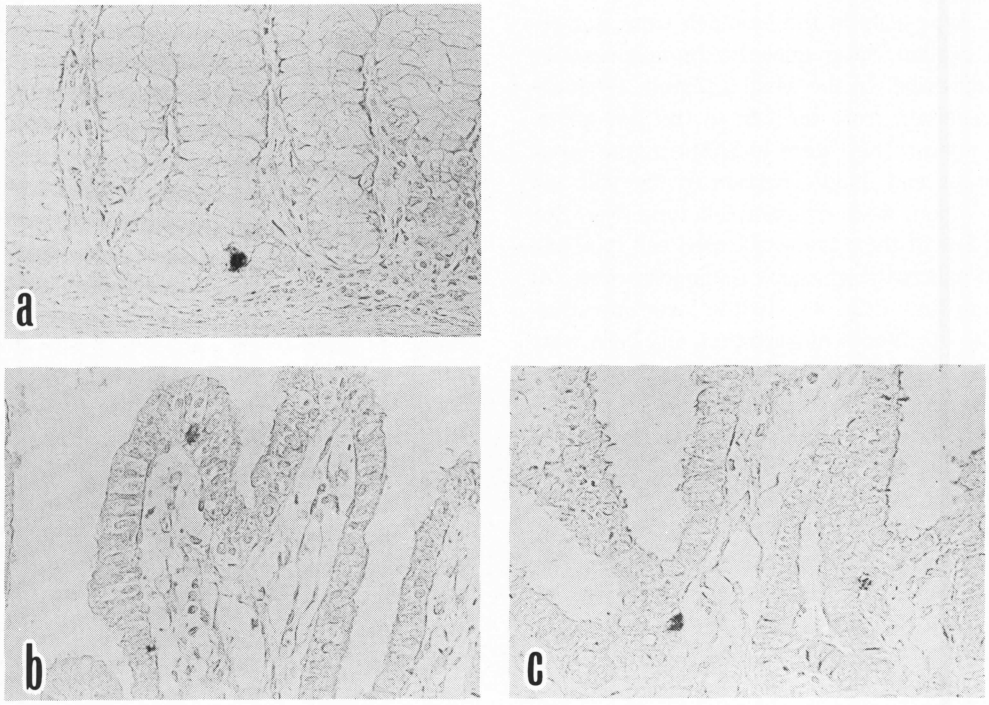

Fig. 6. Little gastrin (a and b) and big gastrin immunostaining in foetal gastrointestinal tract mucosa. (a) Gastrin immunoreactive cells are detected at the base of foetal antral mucosa and show open cell configuration. 5th month of gestation (×126). (b) Gastrin containing cell in Segi's cap and crypt (c) of the small intestine. Serial section from a foetus at the 6th gestational month (both ×126).

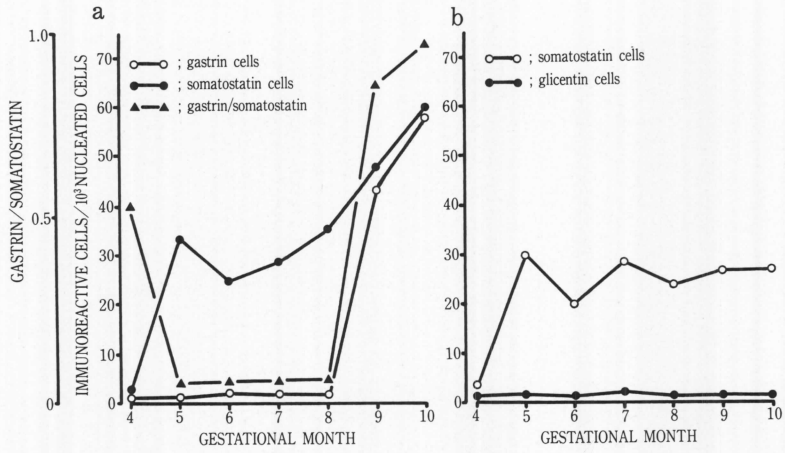

Fig. 7. Chronological changes in the distribution density of gastrin containing cells and somatostatin containing cells in the antrum (a), and glicentin containing cells and somatostatin containing cells in the fundus of the stomach (b).

foetal fundus mucosa were immunoreactive to glicentin C-terminal peptide specific antiserum R4804 from the 5th month of gestation to the last gestational month. Among them, closed type and some open type cells revealed immunoreactivities to glucagon C-terminal specific antiserum OAL123 (Fig. 8a,b). Goblet or brush-bordered intestinal type cells in the stomach were not observed around these glicentin immunoreactive cells (Glic-cells). In the small intestine, Glic-cells were observed from the 5th to the last gestational month. They were localized in the intestinal crypt and middle portion of the villi and most of them were of open cell type (Fig. 8c). Only a few of them were of closed cell type and showed pancreatic glucagon immunoreactivity at the same time (Fig. 8d). In the large intestine, from the 5th month of gestation, Glic-cells were observed at the deep portion of the crypts, middle to sometimes top of the glands (Fig. 8e). The majority of them were open cell type endocrine cells but a few simultaneously showed pancreatic glucagon immunoreactivity (Fig. 8f).

3) Somatostatin

Somatostatin immunoreactive cells (So-cells) were observed from the 4th month of gestation in the foetal gastrointestinal tract mucosa.

In the stomach, they were of open cell type in the antro-pyloric region, whereas in the oxyntic mucosa they were of closed cell type localized in the deep portion of the gastric glands (Fig. 9a). The number of So-cells was greater than that of gastrin or glicentin immunoreactive cells throughout the foetal period (Fig. 7). In the small intestine, So-cells were seen in the intestinal crypts, middle portion of the developing intestinal villi and also in Segi's caps from the 4th month of gestation. All of them showed open cell character in contrast to that of gastric mucosa (Fig. 9b). In the large intestine, So-cells were observed in the crypts and top of the colonic glands. Their configurations were of closed cell type in the former and open cell type in the latter, respectively (Fig. 9c).

4) Motilin

Motilin immunoreactive cells were not observed in the stomach and large intestine throughout the foetal period.

In the small intestine, motilin immunoreactivity was seen in endocrine cells localized in the middle of intestinal villi and Segi's caps from the 5th month of gestation (Fig. 10).

5) Serotonin

In the stomach, EC-cells were observed from the 4th gestational month in the fundic mucosa and from the 5th month of gestation in the antro-pyloric region. They were of closed cell character (Fig. 11a). In the small intestine, serotonin immunoreactivity was observed in the endocrine cells of the crypt, middle portion of the intestinal villi and also Segi's caps. Their configurations were of open cell character in comparison with that of the stomach (Fig. 11b). In the large intestine, EC-cells were observed mainly in the middle to top of the colonic glands, but a few cells were also seen in the crypt. Most

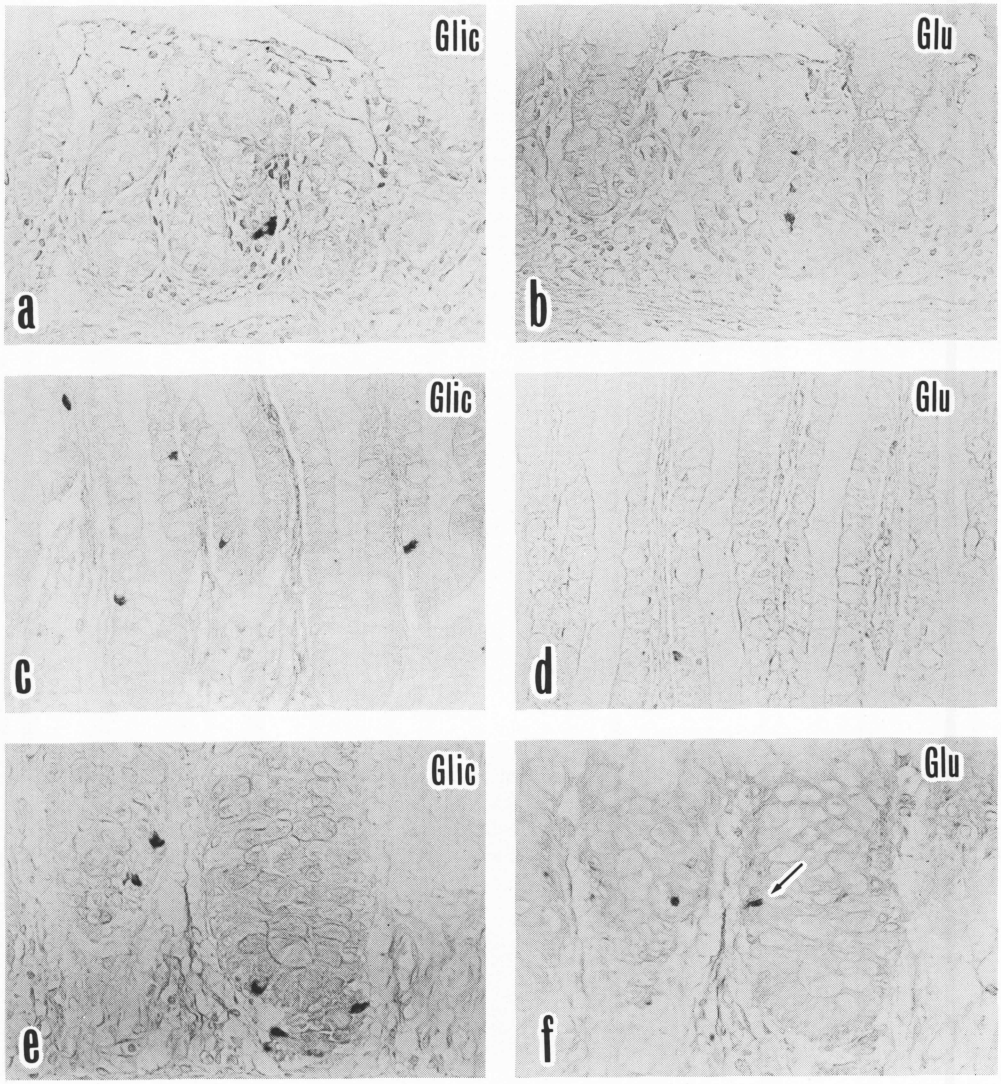

Fig. 8. Glicentin (a, c and e) and pancreatic glucagon (b, d and f) immunoreactivity in the foetal gut. (a and b) Some glicentin immunoreactive cells show pancreatic glucagon immunoreactivity simultaneously. Serial section from foetal oxyntic mucosa. 5th month of gestation (both ×126). (c) Glicentin immunoreactive cells are scattered in foetal small intestinal mucosa and most of them show open cell character. (d) Some of them also present pancreatic glucagon immunoreactivity. Serial section from the foetus at the 10th gestational month (both ×126). (e and f) In foetal large intestine, glicentin containing cells are mainly of open type and some of them show pancreatic glucagon immunoreactivity, but glucagon containing cells not showing glicentin immunoreactivity are also observed (arrow). Serial section from a foetus at the 10th month of gestation (both ×126).

of them were of open cell character but some of them were of closed type (Fig. 11c). EC-cells were not identical to motilin immunoreactive cells in all parts of the foetal gastrointestinal tract throughout the foetal period.

6) Calcitonin

Calcitonin immunoreactivity could not be observed in the gastrointestinal tract mucosa throughout the foetal period.

DISCUSSION

Cationic, antibacterial enzyme lysozyme is well known to be present in the normal gastrointestinal mucosa such as the cardiac and pyloric glands of the stomach, Brunner's duodenal gland and Paneth cells of the small intestine[34,40]. Im-

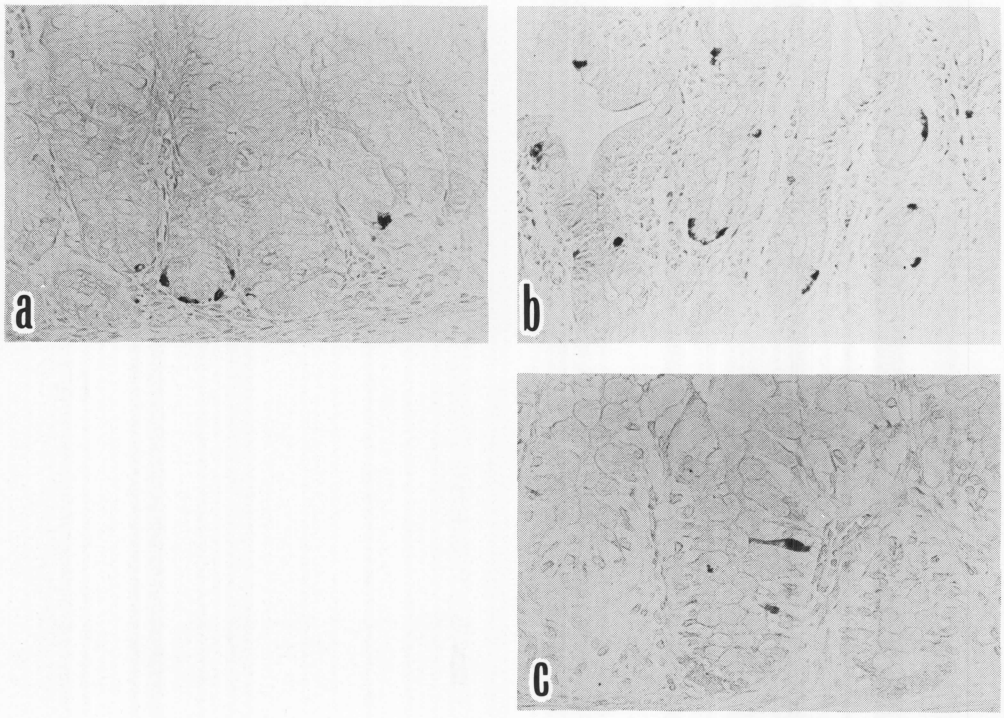

Fig. 9. Somatostatin immunoreactivity in the foetal gut mucosa. (a) Somatostatin containing cells are localized at the base of gastric glands and are of closed cell character. Oxyntic mucosa from the foetus at the 5th gestational month (×126). (b) Somatostatin immunoreactive cells are of open type in foetal small intestine. 10th month of gestation (×126). (c) Flask shaped endocrine cells sometimes show somatostatin immunoreactivity in the large intestine. 10th gestational month (×126).

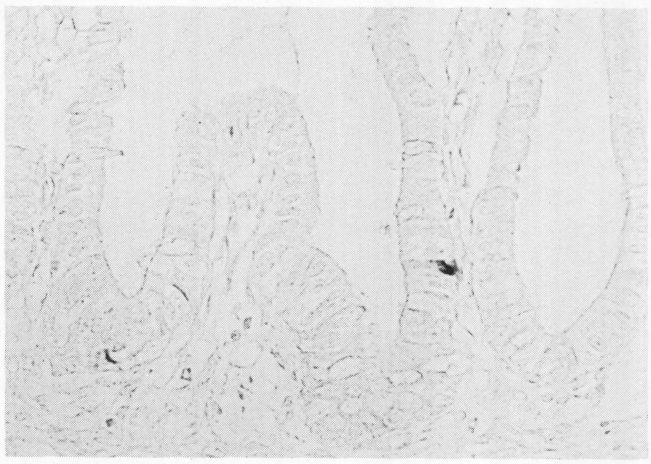

Fig. 10. Motilin containing cells are observed only in the foetal small intestine and are of open cell character. 6th month of gestation (×170).

munoreactive lysozyme was also found in mucous neck cells in chronic gastritis[24]. Tsutsumi et al described that lysozyme might be a marker for proliferative epithelial cells of the stomach because of the presence of its immunoreactivity in the generative zone[64]. Tahara

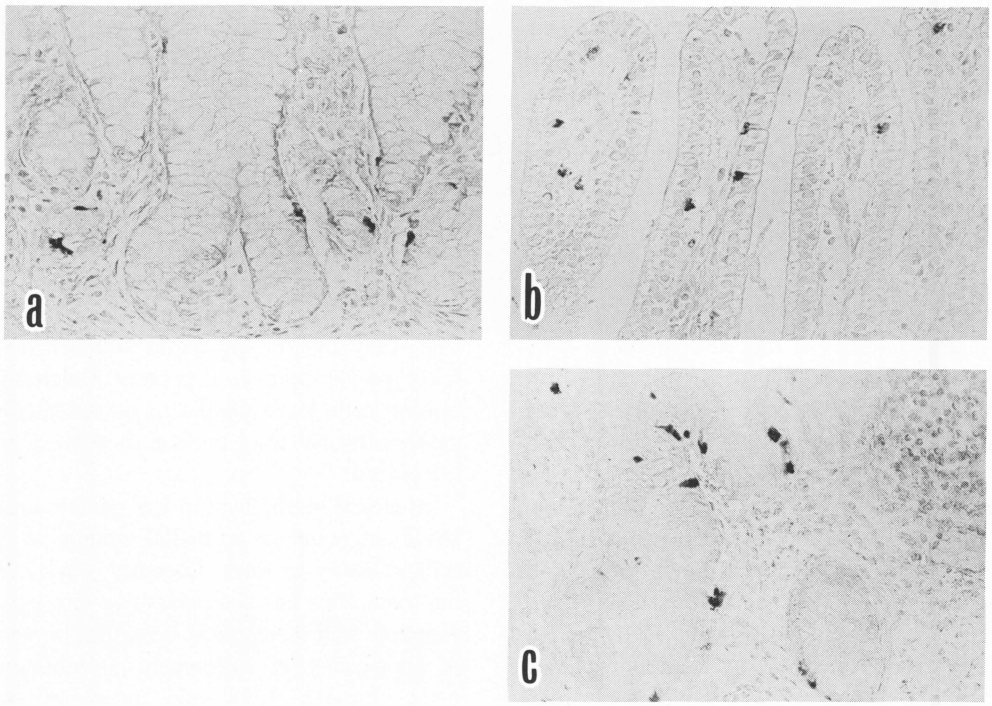

Fig. 11. Serotonin immunoreactivity in the foetal gastrointestinal tract mucosa. (a) Closed type cells reveal serotonin immunoreactivity in the foetal stomach. 5th gestational month (×126). (b) In the small intestine, serotonin immunoreactivity is observed in open type endocrine cells. 6th month of gestation (×126). (c) Both open and closed type endocrine cells show serotonin immunoreactivity. Colonic mucosa from a foetus at the 8th gestational month (×126).

et al also reported that lysozyme immunoreactivity within tumour cells, which was observed in 38% of gastric carcinoma cases, was more intense in deeply invasive carcinomas than in superficially invasive ones[59,60]. However, there are no reports on the ontogeny of lysozyme in human gastrointestinal mucosa. In this study, lysozyme immunoreactivity in the surface epithelium of the foetal stomach reflected the intense proliferative activity of the gastric mucosa in the second trimester[49,51]. On the other hand, in the small intestine lysozyme immunoreactivity was confined to immature Paneth cells and not present in the large intestine throughout the intrauterine period.

Secretory component (SC) is synthesized by epithelial cells of normal small intestine, large intestine and intestinalized gastric mucosa[24,46]. Sumiyoshi et al reported that immunoreactive SC was detected in 87.5% of advanced gastric adenocarcinoma cases of the well differentiated type, which was significantly higher than that of the poorly differentiated type[56]. Although it plays an important role in the local secretory immunity as a receptor and carrier of dimeric IgA molecule[7,63], the development of this glycoprotein in human foetal gastrointestinal tract mucosa has not fully been understood. Tsutsumi et al examined gastric antral mucosa from four foetuses at the 22nd, 27th and 37th gestational week and reported that there was no SC immunoreactivity in the foetal stomach[66]. It was also confirmed in this study that there was no SC immunoreactivity in the developing gastric mucosa from the 4th to 10th month of gestation. Moreover, the author found that immunoreactive SC firstly appeared in crypt epithelial cells of the small and large intestines at the 10th gestational month. In view of the low serum IgA level *in utero*[2] and the role of maternal colostral dimeric IgA ingested after birth in local defense mechanism of the intestine[45], it is reasonable to assume that the expression of SC in the foetal intestinal muco-

sa appears just before birth.

In their original report, Gold and Freedman (1965) described that CEA was present not only in the extractant of several carcinoma tissues but of the foetal gut between the 2nd and 6th month of gestation using immunodiffusion techniques[16]. Since then attention has mainly been focused on the clinical application of CEA as a tumour marker and many investigators reported its immunoreactivity in various cancer tissues[17,46]. However, the precise localization of CEA in the foetal gut has not yet been adequately investigated[43]. Recently, Miyayama and Miyayama studied 10 embryos and foetuses from the 5th to 16th gestational week and showed that CEA immunoreactivity was present in the surface epithelium of the stomach from a foetus at the 16th week of gestation using DAKO polyclonal anti-CEA serum without absorption by splenic powder[42]. Non-absorbed DAKO polyclonal anti-CEA serum detects not only CEA but also CEA related antigens such as non-specific cross reacting antigen (NCA), whereas CEM010 monoclonal antibody used in this study recognizes that an epitope resides in carbohydrate chain portion of CEA molecule and shows no cross-reaction with NCA[22,67]. Ito et al also confirmed the specificity of CEM010 antibody and reported that it reacted with 77.8% of gastric adenocarcinoma cases[26]. Therefore, the earlier results of CEA immunostaining using conventional polyclonal antiserum should be reevaluated. From the present study using monoclonal antibody, "true" immunoreactive CEA in the foetal digestive tract was considered to be localized only in the large intestine between the 6th and 8th month of gestation but not in the stomach or small intestine throughout the intrauterine life.

Alpha-1-antitrypsin (AAT) and alpha-1-antichymotrypsin (ACT) have been reported to be distributed mainly at the base and ascending part of the crypts of the small intestine[15] and pyloric gland of the stomach[33]. In the present study, their distributions in the foetal small intestine were almost identical to those of adult small intestine but they were not observed in the foetal pyloric gland. The expression of AAT and ACT in the adult gastric mucosa might be an acquired phenomenon and is more often expressed in the pyloric gland cells adjacent to gastric adenocarcinoma[61].

In view of the distribution and morphological characteristics of endocrine cells in the foetal digestive tract mucosa in this study, the findings did not significantly differ from the previous reports on those of gastrin[12,36,48], glicentin and pancreatic glucagon[9,14,20,30,38], somatostatin[11,13,47] and motilin[31,35,37,44]. Serotonin containing EC cells are subdivided into EC_1 cells (intestinal type), EC_2 cells (duodenal type) and EC_n cells (gastric type)[52]. It was confirmed in this study that in the foetal stomach only gastric type EC cells were present. Calcitonin containing cells were unable to be detected in the gastrointestinal tract mucosa throughout the foetal period.

Intestinal metaplasia in the gastric mucosa is the most common epithelial change in chronic inflammatory process. Recently, gastric intestinal metaplasia has been classified into two types, complete and incomplete, according to the mode of appearance of sulfomucin containing goblet cells, Paneth cells and intestinal marker enzymes[1,32,41]. In view of endocrine cell population in intestinalized gastric glands, it is characterized by a decrease of gastric-type endocrine cells (gastrin-containing cells and somatostatin-containing cells) and a selective increase of glicentin-containing cells and intestinal-type EC cells[25,27,65]. Since the report of Ascoli (1901)[3], goblet cells and brush-bordered epithelia have been observed in the foetal gastric antrum and cardia[8,49,51]. These intestinal type cells were considered to have a close relationship to intestinalization in adult gastric mucosa[5]. In this study, the existence of these intestinal type cells in the foetal gastric antrum and cardia was also confirmed, of which goblet cells contained PAS and alcian blue positive mucin. However, HID positive sulfomucin containing goblet cells, Paneth cells, SC and CEA immunoreactivity could not be detected in these cells. Any intestinal type endocrine cells containing glicentin or open type EC cells also could not be found around these heterotopical intestinal type cells. These results indicate that intestinal type cells in the foetal gastric mucosa are different from those of intestinal metaplasia in adult gastric mucosa. That is, intestinal metaplasia of the human gastric mucosa is not a fetalization but can be regarded as an acquired phenomenon of

metaplastic change.

HID-positive sulfomucin[18,39], immunoreactive lysozyme[59,60], SC[24,46,56], protease inhibitors[33,61] and CEA[17,46,58] are well known to be present in tumour cells of gastric adenocarcinoma. Moreover, polypeptide hormones and amine frequently occur in ordinary carcinoma of the stomach[4,21,28,57–59,69]. The occurrence of peptide hormones in gastric adenocarcinoma is more closely related to age rather than to site of tumour. When the frequency of peptides in poorly differentiated adenocarcinomas is compared between young patients (under 30 years old) and aged patients (over 70 years old), the frequency of somatostatin is significantly higher in aged patients than young ones, whereas that of gastrin, glicentin and calcitonin is evidently higher in young patients[62]. Moreover, gastric endocrine cell carcinoma evidently occurs in younger patients (30 to 40 years old) and produces more multiple peptide hormones and amines synchronously, compared with that in middle aged patients[58,59,62].

Bosman et al reported that endocrine cell population in gastric adenocarcinoma was almost the same as that of foetal and normal gastric mucosa[6]. However, the incidence of gastrin and somatostatin in gastric carcinoma differs by histological types[62]. For example, in well differentiated adenocarcinoma, the incidence of gastrin and somatostatin is identical (17.2%) and the ratio of gastrin to somatostatin is 1 to 1. This may correspond to the ratio in the number of gastrin producing (G) cells to somatostatin producing (D) cells in the foetal antral mucosa at the last month of gestation. On the other hand, in poorly differentiated adenocarcinoma, gastrin and somatostatin are detected in 13% and 20%, respectively, and its ratio is 1 to 1.5. The ratio in the tumour may correspond to that of the number of G cells to D cells in the foetal antral mucosa at the 6th or 7th month of gestation.

The present results suggest that the expression of SC, CEA, AAT, ACT and sulfomucin or calcitonin in gastric adenocarcinoma cannot be explained merely by the reversion of the tumour cells to the foetal phenotype of the stomach, because these tissue markers, sulfomucin and calcitonin, are not detected in the foetal gastric mucosa. This might be one of the evidences that a wider range of genes in tumour cells is expressed and contributes to the functional dysdifferentiation, although they simulate more or less the morphological characteristics of their originating tissues.

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