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Image Quantitation of Intestinal Metaplasia in Entire Gastrectomy Specimens from Swedish and Japanese Patients

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The aim of this work was to investigate the extension of intestinal metaplasia (IM), as well as to quantitate various components of IM (namely sialomucins, sulfomucins and Paneth cells), in entire gastrectomy specimens from Swedish and Japanese patients. The length of the gastric mucosa was assessed by morphometry. The percent of sections with IM was regarded as the extension of IM in the specimens. Histochemically labeled sialomucins, sulfomucins and Paneth cells (the 3 main findings in gastric IM) were quantified in separate sections with the aid of an image analyzer. In total, 1,321 sections corresponding to 6 gastrectomy specimens were quantified. Sialomucins and sulfomucins were more extensively distributed in the 4 specimens with carcinoma than in the 2 without carcinoma (one having a peptic ulcer and the other, hereditary gastric cancer syndrome (HGCS) without carcinoma). On the other hand, quantitative analysis in Swedish specimens indicated that the highest values for sialomucins, sulfomucins and Paneth cells were present in HGCS. When Swedish and Japanese specimens with adenocarcinoma were compared, only sulfomucins (denoting Types II and III IM) were significantly higher in those carrying an intestinal-type carcinoma (ITC) than in those with diffuse-type carcinoma (DTC). The results substantiate those obtained with gastric biopsies by other authors. On the other hand, the mucosal extension and the amount of sulfomucins are not comparable parameters (since that mucin was not equally distributed, but "concentrated" in certain areas in the mucosa). One possible conclusion is that the focal distribution of acidic mucins and of Paneth cells in the gastric mucosa may strongly influence their detection rate in gastric biopsies. Thus, haphazard biopsy of the gastric mucosa may fail to sample areas with sulfomucins in population studies aiming to detect individuals at risk. Such sampling errors in gastric biopsies may explain the conflicting results on this subject appearing in the literature.

Key words: Quantification — Gastric mucosa — Intestinal metaplasia — Cancer

Despite decreasing trends in the incidence of gastric carcinoma, the mucosa of the stomach remains one of the most frequent targets for cancer development throughout the world.[1] The etiology of gastric carcinoma remains uncertain. However, several histologic alterations of the gastric mucosa appear to be related to the pathogenesis of gastric carcinoma since they antedate or cooccur with that lesion. Those alterations are chronic (atrophic) gastritis,[2] dysplasia,[3] intestinal metaplasia (IM),[4] adenomatous polyps[5] and intramucosal cysts.[6,7] One of them, IM, has received much attention in the literature and is today accepted to be a precursor of gastric carcinoma.[2,8–15] Several theories have been proposed to explain the possible role of IM in the pathogenesis of gastric carcinoma. One of them concerns the histologic type of IM. This lesion has been histologically classified into complete and incomplete.[3,13,14] Several workers have found that stomachs with IM of incomplete type are more prone to develop carcinoma.[3,13] For others,[15,16] it is the extension of IM in the gastric mucosa that is of more significance. Using quantitative morphometry in gastrectomy specimens, it was found that IM is more extensively distributed in the gastric mucosa of the Japanese,[17] Maories[18] and Chileans[12] (i.e., populations at high risk to develop gastric carcinoma) than in Swedes and Mexicans[10,19] (populations at a much lower risk to develop gastric carcinoma). A third group of researchers[20–22] consider that the important factor is chemical alteration of the acidic mucins (sialomucins and sulfomucins) found in the metaplastic epithelium. Sialomucins are stained by alcian blue at pH 2.5 (AB) and sulfomucins by high iron diamine (HID). Using a composite staining procedure (AB-PAS-HID), three histochemical-histological types of IM have been described[22]: Type I, in which goblet cells are stained with AB, contains columnar absorptive cells and Paneth cells; Type II in which goblet cells are stained with AB and sometimes with HID, contains columnar cells with non-sulfated mucous secretion and lacks Paneth cells; and Type III, in which the goblet cells react as in type II, the columnar cells have HID-positive mucous secretion and Paneth cells are absent. Some authors claim that patients having

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type III IM are at high risk to develop gastric carcinoma.[21, 23]

We have investigated all three aforementioned theories.[10, 12, 18, 19, 24–28] But those studies were done separately in different countries using different instruments and quantification programs. The results obtained are therefore not totally comparable. We have now analyzed both the extension and the histochemical components of IM in gastrectomy specimens from Swedish and Japanese patients using the same image analyzer and the same computer program of quantification.

MATERIALS AND METHODS

In total, 1,321 sections were studied from 6 gastrectomy specimens. The method for sectioning the entire specimen has been reported elsewhere.[27] After fixation, the entire specimen was cut into blocks. Four consequtive sections from each block were stained with hematoxylin and eosin (HE), AB, HID and acid fucsin (AF). No counterstains were used.

Quantification of AB, HID and AF positive cells Each section was analyzed in an image quantifier (Fig. 1). The set-up consists of a light microscope (Leitz-Weztlar, Germany) connected to a video camera (Ikegami, Tokyo) which transfers the image to a TV monitor operated via a Macintosh computer (LC II), programmed with a quantifier program (Optilab 2.01, Paris). The program processed the blue color (of the AB stain), the various shades of brown-black (of HID) and the red colour (of AF) into a 256 grade color scale. The remaining unstained mucosa could be easily discriminated on the screen.

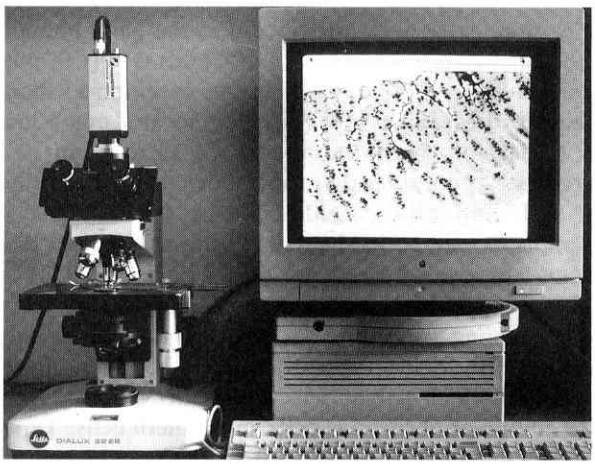

Fig. 1. Image quantifier device (light microscope-video camera, Ikegami, Tokyo) which transfers the image to a TV monitor operated via a Macintosh computer (LC II), programmed with a quantifier program (Optilab 2.01, Paris). The screen shows gastric mucosa stained with high iron diamine (HID) (without counterstain) to reveal sulfomucin.

All sections were analyzed using a 6.5× objective. Using the appropriate threshold, the percentage of histochemically labeled cells was automatically calculated and displayed on the screen. All consecutive fields in every section (which included the entire mucosal thickness) were quantified.

The length of the mucosa in gastrectomy specimens was assessed by morphometry.[24] Different indices of IM were calculated for AB, HID and AF by using the formula:

$$\text{Index} = \frac{\text{percent of histochemically labeled cells}}{\text{total length of mucosa}}$$

Statistical analysis: One-way ANOVA was used to test the significance of differences.

RESULTS

The patients Four of the 6 patients were Swedish and the remaining 2 were Japanese. All 6 patients were males. Of the 4 Swedish patients, one had a gastric peptic ulcer (GPU), one intestinal-type carcinoma (ITC), the third diffuse-type carcinoma (CDT) and in the fourth no tumor or ulcer was found, but a preoperative gastric biopsy had demonstrated low-grade dysplasia. This last patient wished to be operated upon because 5 members of his family had died of gastric carcinoma (hereditary gastric cancer syndrome, HGCS). Of the 2 Japanese specimens, one had ITC and the other DTC. The age of the Swedish patients ranged from 65 to 69 years. The 2 Japanese patients were 56 and 58 years old.

The specimens

Total number of sections: Of the total of 1,321 sections, 442 (mean 73.8, range 55–100 sections) were stained with AB, 432 sections (mean 73.8, range 50–100 sections) with HID and 447sections (mean 74, range 57–102 sections) with AF.

Total mucosal length/gastrectomy: A total length of 4,906.4 cm of gastric mucosa from the 6 gastrectomy specimens was morphometrically analyzed. The length analyzed in the 442 sections stained with AB was 1,639.1 cm (mean 273.2 cm/gastrectomy, range 203.5–370.0 cm), in the 432 sections stained with HID, 1,624.5 cm (mean 273.2 cm/gastrectomy, range 203.5–370.0 cm) and in the 447 stained with AF, 1,642.8 cm (mean 273.8 cm/gastrectomy, range 210.9–370.0 cm). The difference was not significant ($P < 0.6$).

Total number of slides with histochemically labeled cells: The results are summarized in Table I. Of the 442 sections stained with AB (Fig. 2), 321 sections or 72.5% (range 56–100%) had one or more AB-labeled foci. Of

Table I. The Distribution of Alcian Blue (AB), High Iron Diamine (HID) and Acid Fucsin (AF) Stains (in Percent of Slides with Stained Cells in the Entire Specimen) in 6 Gastrectomy Specimens: 4 in Swedish Patients and the Remaining 2 in Japanese Patients

Histology			Percent sections		
			AB	HID	AF
Adenocarcinoma	Intestine	(Swede)	70 (42/60)	82 (49/60)	38 (23/60)
	Diffuse	(Swede)	97 (66/68)	94 (61/65)	96 (65/68)
Peptic	Ulcer	(Swede)	40 (30/76)	40 (30/76)	1 (1/76)
Hereditary	Carcinoma	(Swede)	56 (46/82)	54 (45/83)	60 (50/83)
Adenocarcinoma	Intestine	(Japanese)	100 (58/58)	98 (49/50)	76 (44/58)
	Diffuse	(Japanese)	94 (92/98)	90 (88/98)	88 (90/102)
Total			75.6 (334/442)	74.5 (322/432)	61.1 (273/447)

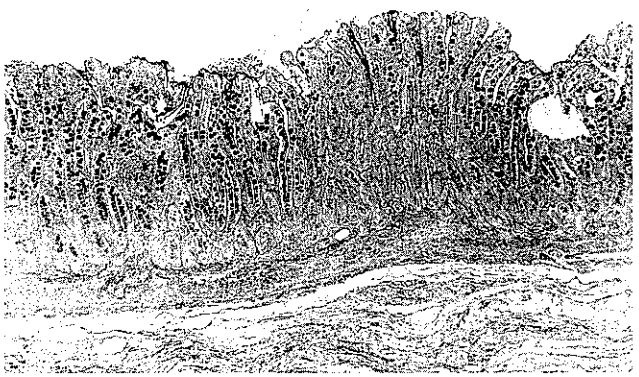

Fig. 2. Gastric mucosa with goblet cells stained with Alcian Blue, pH 2.5 (without counterstain) to reveal sialomucin (× 65).

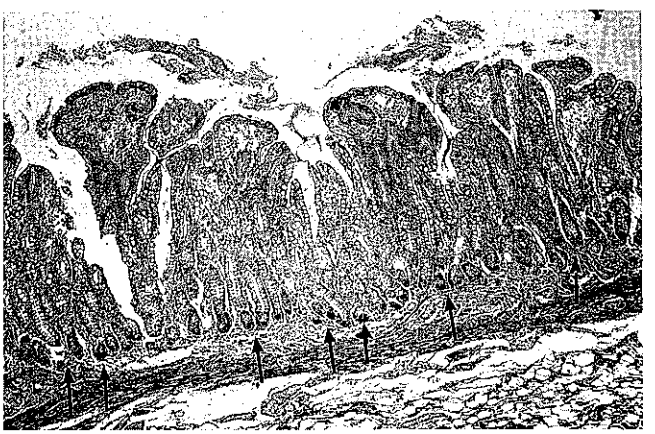

Fig. 3. Gastric mucosa stained with acid fucsin (without counterstain). Paneth cells are indicated by arrows (×65).

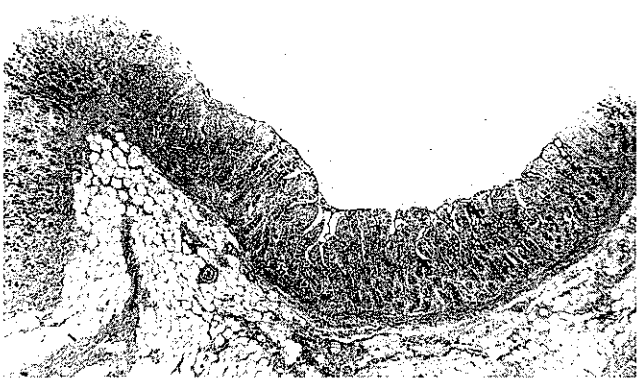

Fig. 4. Gastric mucosa stained with high iron diamine (without counterstain) to visualize sulfomucin. Note focal staining (×25).

the 432 sections labeled with HID 323 sections or 73.6% (range 40–98%) had one or more HID-labeled foci and of the 447 sections stained with AF (Fig. 3), 274 or 61.7% (range 1–96%) had one or more AF-labeled foci. The difference between AB and HID was not significant ($P<0.6$). On the other hand, the AB and HID values were significantly higher than the AF values ($P<0.04$).

Quantification of histochemically labeled cells: The total positive AB staining recorded in the 6 gastrectomies (Table II) was 12,758.1 (mean 2,126.4/gastrectomy, range 297.2–3,816.6). For HID (Table III), it was 10,309.8 (mean 1,718.3 /gastrectomy, range 303.6–2,740.8) and for AF (Table IV), 2,640.6 (mean 440.1 / gastrectomy, range 1.0–767.6). The difference between AB and HID was not significant ($P<0.6$), but the AB and HID values were significantly higher than the AF values ($P<0.04$).

Histochemical indices: The AB, HID and AF histochemical indices (i.e., the values recorded for histochemically

Table II. The Alcian Blue (AB) Index (i.e., the Length of the Gastric Mucosa in the Entire Specimen/Quantified AB values in the Full Thickness of the Mucosa in the Entire Specimen) in 6 Gastrectomy Specimens: 4 in Swedish Patients and the Remaining 2 in Japanese Patients

Histology			Length (cm)	Quantified AB	AB index (/cm)
Adenocarcinoma	Intestine	(Swede)	222.0	1,809.5	8.2
	Diffuse	(Swede)	259.0	1,583.1	6.1
Peptic	Ulcer	(Swede)	281.2	297.2	1.1
Hereditary	Carcinoma	(Swede)	303.4	3,743.8	12.3
Adenocarcinoma	Intestine	(Japanese)	203.5	1,507.9	7.4
	Diffuse	(Japanese)	370.0	3,816.6	10.3
Total			1,639.1	12,758.1	45.4

Table III. The High Iron Diamine (HID) Index (i.e., the Length of the Gastric Mucosa in the Entire Specimen/Quantified HID in the Full Thickness of the Mucosa in the Entire Specimen) in 6 Gastrectomy Specimens: 4 in Swedish Patients and the Remaining 2 in Japanese Patients

Histology			Length (cm)	Quantified HID	HID index (/cm)
Adenocarcinoma	Intestine	(Swede)	222.0	1,447.7	6.5
	Diffuse	(Swede)	259.2	983.3	3.8
Peptic	Ulcer	(Swede)	281.2	303.6	1.1
Hereditary	Carcinoma	(Swede)	307.1	2,372.0	7.7
Adenocarcinoma	Intestine	(Japanese)	185.0	2,462.4	13.3
	Diffuse	(Japanese)	370.0	2,740.8	7.4
Total			1,624.5	10,309.8	39.8

Table IV. The Acid Fucsin (AF) Index (i.e., the Length of the Gastric Mucosa in the Entire Specimen/Quantified AF in the Full Thickness of the Mucosa in the Entire Specimen) in 6 Gastrectomy Specimens: 4 in Swedish Patients and the Remaining 2 in Japanese Patients

Histology			Length (cm)	Quantified AF	AF index (/cm)
Adenocarcinoma	Intestine	(Swede)	222.0	212.8	0.9
	Diffuse	(Swede)	251.6	595.2	2.4
Peptic	Ulcer	(Swede)	281.2	1.0	0.03
Hereditary	Carcinoma	(Swede)	307.1	767.5	2.5
Adenocarcinoma	Intestine	(Japanese)	210.9	311.8	1.5
	Diffuse	(Japanese)	370.0	752.2	1.9
Total			1,642.8	2,640.5	9.23

labeled cells/ total length of mucosa) in the 6 specimens are shown in Tables II-IV. The mean AB index (Table II) was 7.6 (range 1.1–12.3). The highest AB index was recorded in a Swedish specimen with HGCS (12.3), followed by a Japanese specimen with DTC (10.3). The lowest AB index was found in a Swedish specimen with a GPU (1.1). The mean HID index (Table III) was 6.6 (range 1.1–13.3). The highest HID index was recorded in a Japanese specimen with ITC (13.3) followed by a specimen with HGCS (7.7), the lowest value being found in the specimen with GPU (1.1). The mean AF index (Table IV) was 1.5 (range 0.03–2.5). The difference between AB and HID mean indices was not significant ($P < 0.6$), but the AB and HID indices were significantly higher than for AF ($P < 0.04$). The highest AF index was recorded in a Swedish specimen with HGCS (2.5), followed by a Swedish specimen with DTC (2.4), the lowest index being found in the Swedish specimen with GPU (0.03).

Histochemical indices in specimens from Japanese and Swedish patients with gastric carcinoma: The HID index was significantly higher ($P < 0.04$) in the Japanese speci-

mens with ITC and DTC than in the Swedish ones of comparable histologic types. The AB index was significantly higher ($P<0.04$) in the Japanese specimen with DTC than in the Swedish with the same tumor phenotype. No difference in the AB index was found in the Swedish and the Japanese specimens with ITC.

Although the AF index was somewhat higher in the Japanese specimen with ITC than in the Swedish specimen with the same histologic phenotype, the difference was not significant ($P<0.6$).

DISCUSSION

Based on the alkaline phosphatase activity of the surface gastric mucosa some authors have estimated the distribution of IM in gastrectomy specimens.[15, 29] However, as Stemmermann and Hayashi pointed out,[15] enzymatic and microscopic examination are roughly comparable. In fact, studies on frozen sections indicated that enzymatic staining (to demonstrate alkaline phosphatase) was limited to the cell surface. When the IM mucosa was covered by histologically normal surface epithelium, IM remained undetected by that enzymatic stain. Conversely, some metaplastic zones were unstained by the enzymatic assay. Another pitfall of the method was that enzyme-treated preparations could not be stored or reevaluated.

By the method described here, we have quantified the various components of IM in the entire mucosal thickness in sections from gastrectomy specimens without and with carcinoma. The same image analyzer program (Optilab 5.0) was used to analyze all specimens. It may be argued that the number of cases measured is small. This is partly because the method used here is time-consuming: to quantify the histochemical reactions in the 1,321 sections took seven months to complete for a specialized technician working full-time (M.M.). On the other hand, this study has provided the first systematic quantitative analysis in the literature of the various components of IM in entire gastrectomy specimens.

The study of the percent of sections with positive AB staining indicated that sialomucin was more extensively distributed in the Swedish specimen with DTC than in the one with ITC, whereas in the Japanese, sialomucins were similarly extended in DTC and ITC. The specimens with HGCS and GPU showed a less extensive sialomucin distribution than specimens with carcinoma. Thus sialomucins were more widely distributed in gastrectomy specimens with carcinoma than in those without carcinoma, thus confirming our previous morphometric studies in HE-stained sections from entire gastrectomy specimens.[16, 17, 24, 25] On the other hand, quantitative measurements demonstrated that the total amount of sialomucin was highest in the Swedish patient with HGCS. Lower values were found in the 2 Japanese specimens with carcinoma and in the Swedish specimens with carcinoma and peptic ulcer. Thus, sialomucin production was highest in a stomach without carcinoma (i.e., HGCS). On the other hand, all 4 stomachs with carcinoma and the one at risk to develop gastric carcinoma (HGCS) had much higher sialomucin values than the patient with GPU, suggesting that the focal production of sialomucins may be increased in the gastric mucosa of patients at risk or harboring a gastric carcinoma. The results also demonstrated that the extension and the amount of sialomucin in the gastric mucosa were not comparable (since mucin was unequally distributed or "concentrated" in certain mucosal areas).

The study of the percent of sections having positive HID staining indicated that sulfomucin was more extensively distributed in stomachs with carcinoma than with HGCS or GPU. On the other hand, the mucosal extension of sulfomucin was similar in the 4 specimens with gastric carcinoma (somewhat lower in the Swedish specimen with ITC). Quantitative analysis indicated, however, that the total amount of sulfomucin was higher in Japanese and Swedish specimens with ITC than with DTC. Since sulfomucin labels Types II and III IM,[30] light microscopy[18] was necessary to demonstrate that the HID-positive substance occurred in both goblet and columnar cells (i.e., Type III IM) in all 4 specimens with carcinoma, particularly in ITC. The finding that sulfomucin was increased in specimens carrying ITC seems to substantiate the results obtained by non-quantitative visual estimations of histochemically labeled gastric biopsies in some publications.[21–23, 30, 31] The results also indicated that the extension and the amount of sulfomucin in the gastric mucosa were not comparable (since mucin was not equally distributed, but "concentrated" in certain areas in the mucosa).

The study of the percent of sections having positive AF staining indicated that Paneth cells were widely distributed in Swedish and Japanese specimens with ITC and DTC. Not only was the distribution of Paneth cells wide in specimens with ITC and DTC, but also the total amount of those cells was higher in carcinomas. Thus, Paneth cells did not protect those individuals from gastric cancer growth as suggested in the literature.[23]

Based on visual impression, several authors claimed that IM develops around gastric tumors or benign ulcers. We also found in this work the occurrence of IM in the mucosa surrounding those lesions. However, the quantitative assessment revealed that in all 6 specimens the indices of sialomucins and sulfomucins were highest in at least one of the sections away from the tumor area or from the benign ulcer. It appears, therefore that IM does not preferentially develop about gastric carcinomas or ulcers, an opinion shared by some authors (Y. Kato, personal communication).

Which is the important additional information provided by the different histochemical stains other than HE? AB stains goblet cells, which are seen in Type I IM and in the majority of types II and III IM[21, 23] (i.e., complete and incomplete IM). AF stains the Paneth cells present in Type I IM.[23, 27] But as goblet and Paneth cells are readily observed in HE-stained sections, AB and AF stains provide no additional information (over HE stain) and are of questionable value in classifying IM as proposed in the literature. PAS is used to differentiate Type II from Type I IM.[21–23] But as only Type III is claimed to be of significance in gastric carcinogenesis,[22, 30] PAS stain also appears as superfluous in detecting type III IM. In the present work, HID was the only stain that gave additional information: it detected increased amounts of sulfomucin in specimens with carcinoma, particularly ITC.

From the results it is clear that the focal distribution of acidic mucins and of Paneth cells in the gastric mucosa may strongly influence their detection rate in gastric biopsies. Since sulfomucin was widely distributed in ITC, DTC and HGCS and it was found even in GPU, its presence in gastric biopsies may not necessarily indicate increased risk for gastric malignancy in population analysis (Fig. 4). Conversely, sulfomucins were absent in areas with IM in specimens with ITC and DTC (despite the abundance of sulfomucin in many other mucosal areas elsewhere in the specimens). Another possible conclusion from these findings is that haphazard biopsy of the gastric mucosa may fail to sample areas with sulfomucins in population studies aiming to detect individuals at risk. The unavoidable sampling error in gastric biopsies may explain the conflicting results on this subject appearing in the literature.[32–35]

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