

Chronological Evaluation of Histologic Changes in the Healing of Benign Gastric Ulcers: Morphologic Indicators of the age of the Ulcer

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Summary. Histologic changes in the healing process of gastric ulcers were studied in relation to their age, i.e., the time period between the onset of an ulcer and gastrectomy. Observation was made in 27 stomachs with 31 oval ulcers or scars. Newly formed glands occurring in the regenerative mucosa with a tuft configuration, tended to occur at an ulcer age of one month or more. The regenerative mucosa regained a normal histologic appearance in 3 months. Histologic features of the submucosal fibrosis surrounding the ulcer also sequentially changed after the ulcer's onset: initially from edematous fibrosis with activated fibroblasts (F1), through dense and tight fibrosis (F2), and finally to thin and loose fibrosis (F3). F1-, F2-, and F3-fibrosis approximately indicated an ulcer age of one month or less, one to 12 months, and one year or more, respectively. In the healing process of deep ulcers involving the muscularis propria, the muscularis mucosae fused together with the muscularis propria at the ulcer margin between 1.2 and 5.4 months after the ulcer's onset. The state of submucosal fibrosis surrounding the ulcer, presence or absence of the fusion of the two muscle layers at the ulcer margin, as well as the features of the regenerative epithelium covering the ulcer base are considered reliable histologic indicators of the age of a gastric ulcer.

INTRODUCTION

The process of repair of gastric ulcers has been enthusiastically studied using various methodologies including x-ray,¹⁾ endoscopy,²⁻⁴⁾ stereoscopic microscopy,⁵⁾ histology,⁶⁾ histochemistry,⁷⁾ and autoradiography.⁸⁾ The great majority of previous reports morphologically investigating the healing course of

gastric ulcer concerned histologic changes in the regenerative epithelium of the gastric mucosa covering the ulcer base, with, however, only a few of these dealing with those histologic changes in relation to the time required for the development of such changes after initial ulceration of the stomach.⁶⁾ Moreover, histological changes sequentially occurring in the submucosal tissue around the ulcer have not been well documented as yet. The purpose of the present study was, therefore, to evaluate the histological changes in the regenerative epithelium of the gastric mucosa covering the ulcer base and in fibrosis developing in the submucosa around the ulcer in relation to the time passing after the onset of ulcer, and to determine reliable histologic indicators of the age of the gastric ulcer.

MATERIALS AND METHODS

Records of gastrectomy specimens in the First Department of Pathology at Niigata University Hospital during the period from January 1980 till December 1986 were reviewed to find stomachs with benign gastric ulcers or scars meeting the following criteria: 1) an oval-shaped ulcer not accompanied by preceding ulcer scars at the ulcer margin, or an oval-shaped ulcer scar with concentric convergence of the gastric mucosa; 2) cases in which an ulcer's age was defined as the time between endoscopic identification of the acute (or active) stage of the ulcer in terms of the staging system for ulcer healing reported by Sakita et al.²⁾ and resection of the stomach with the ulcer was available for the study. As a result, 27

stomachs with 31 foci of peptic ulcers or scars were identified out of 5537 gastrectomy specimens. Of the 27 stomachs, 4 stomachs each had two lesions of ulcers or scars, while the other 23 stomachs had one lesion.

The ages of the 27 patients with such lesions ranged from 19 to 85 years, with a mean age of 56 years; male/female ratio was 19:8. The ages of the ulcers ranged from 2 to 1460 days, with a median age of 42 days in the 27 patients. The number of endoscopic or x-ray examinations performed in these patients to detect the active stage of the ulcer and subsequently to follow up the healing of the ulcer ranged from one to six with a median number of two. No ulcer relapse was evident in any of the cases during the follow-up. Twenty-six of the 27 stomachs were surgically resected specimens. Gastrectomy was performed in these 26 patients for various reasons such as imminent perforation, uncontrollable bleeding from the ulcer, shock, or later detection of gastric cancer developing at different sites from benign ulcers or scars during the follow-up. The remaining one stomach was obtained at autopsy performed on a patient succumbing to carcinoma of the liver.

Of the 31 lesions, 17 were open ulcers for which the base was not completely covered by a regenerative epithelium of the gastric mucosa, and 14 were a healed ulcer i.e., ulcer scar. The greatest diameter of the ulcers or scars ranged from 2 to 20 mm with a median diameter of 7 mm. Two lesions were located in the proximal third of the stomach, 28 were in the middle third, and the remaining one was in the distal third. The muscularis propria was intact in 11 and partially or completely destroyed in 20 lesions.

The resected specimens were opened longitudinally and stretched on a board immediately after resection. After fixation in 10% formalin, each ulcer or scar together with all the spread of the surrounding fibrosis in the stomach wall was step-sectioned at 4 mm for blocking (Fig. 1-A), and all of the rectangular tissue blocks were processed and embedded in paraffin wax. Sections were routinely stained by hematoxylin and eosin. Azan-Mallory stain was added in some cases for discrimination between muscle and collagen fibers.

Histologic changes in the regenerative epithelium at the ulcer or scar and those in the submucosal fibrosis surrounding the ulcer were evaluated in relation to the ulcer's age for all cases as illustrated in Fig. 1-B. In addition, the presence or absence of the fusion of the muscularis mucosae with the muscularis propria at the ulcer or scar and the time required for the occurrence of the fusion were also assessed. In

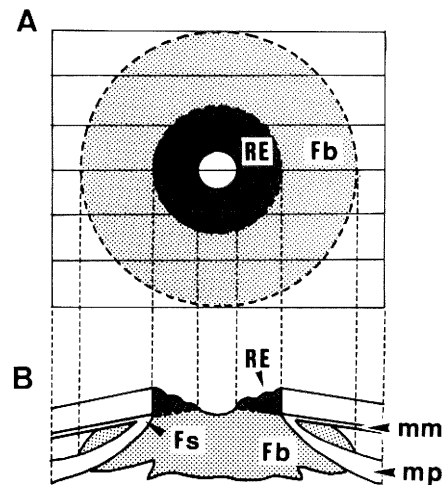


Fig. 1. Step-sectioning of the specimen (A) and schematic drawing of the histologic features in an ulcer (B). RE: regenerative epithelium, Fb: fibrosis, Fs: fusion of muscle layers, mm: muscularis mucosae, mp: muscularis propria.

the present study, newly formed i.e., regenerative epithelium or mucosa was defined as that present on the area of the disruption or disarrangement of muscularis mucosae. Histologic changes of the regenerative mucosa covering the ulcer base were evaluated at the maturest part of the regenerated mucosa.

RESULTS

1. Sequential changes of the histologic features of the regenerative epithelium

Histologic features of the regenerative epithelium or mucosa could be classified into the following three stages based on the presence or absence of glandular regeneration and the degree of the maturity of the glands in the regenerative mucosa: an immature stage at which the regenerative epithelium had a membranous or papillary appearance without regeneration of the mucous glands resembling those seen in the antral mucosa of the stomach (pseudopyloric glands); a juvenile stage at which the regenerating mucosa was usually tufted in appearance with a few pseudopyloric glands newly formed (Fig. 2); and a mature stage indicating the regenerative mucosa having as many glands as those in the neighbouring

mucosa. Accordingly, the regenerative mucosa at the immature stage was found in 10, that at the juvenile stage in 8, and that at the mature stage in 11 of the 31 lesions of ulcers or scars. Epithelial regeneration was not yet recognized at the ulcer's margin in the other two lesions.

The time taken for the regenerating mucosa to develop into each of the stages summarized in Table 1. All of the ulcers with a regenerative epithelium corresponding with the immature stage had an ulcer age of one month or less. The regenerating mucosa at the juvenile stage appeared from 0.8 to 2.8 months

Table 1. Histologic features of the regenerative epithelium in relation to the age of gastric ulcer

Regenerative epithelium	n	Ulcer age (months)	(Median)
Immature stage	10	0.1- 1.0	(0.7)
Juvenile stage	8	0.8- 2.8	(1.6)
Mature stage	11	2.6-48	(10.5)

after the ulceration. All of the ulcers repaired by the regenerative mucosa at mature stage had an ulcer age of 2.6 months or more. Nine (90%) of the 10

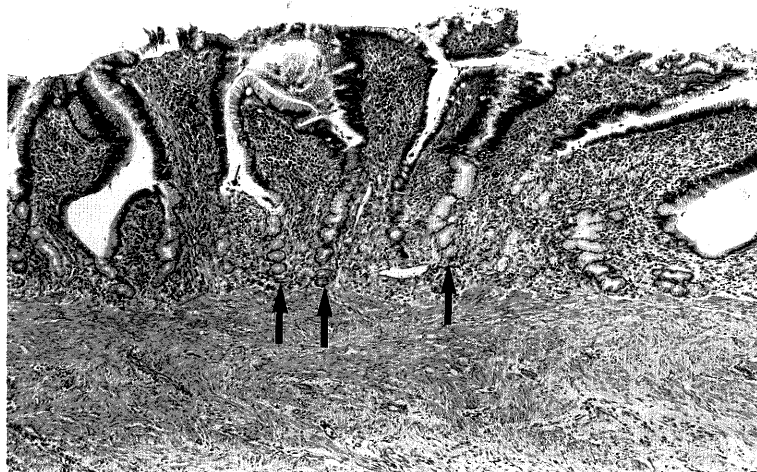


Fig. 2. Regenerative mucosa at the juvenile stage with a tufty appearance. Pseudopyloric glands (arrows) are newly formed at this stage. Hematoxylin and eosin stain. $\times 16$

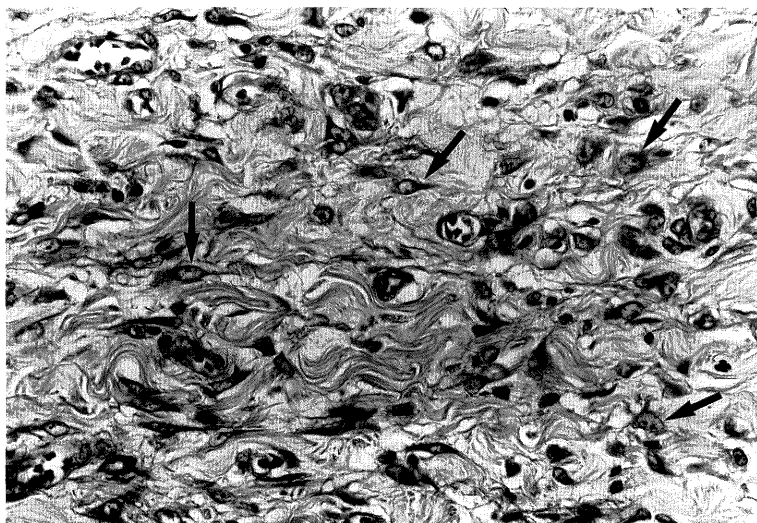


Fig. 3. F1-fibrosis. Note the fine waves of collagen fibers and numerous activated fibroblasts (arrows). Hematoxylin and eosin stain $\times 80$

ulcers having a regenerative epithelium at the immature stage still represented open ulcers; six (75%) of the 8 ulcers having that at the juvenile stage remained open; and all of the 11 ulcers having that at the mature stage were healed ulcers, i.e., ulcer scars. The emergence of parietal cells, chief cells, and goblet cells was observed in the regenerating mucosa at the mature stage: parietal cells in three, chief cells in one, and goblet cells in two ulcer scars.

2. Sequential changes of the histologic features of submucosal fibrosis around the ulcer

Histologic features of submucosal fibrosis occurring around the ulcer could be classified into the following three types based on the appearance of collagen fibers and fibroblasts: F1-fibrosis characterized by the presence of fine waves of collagen fibers in the edematous stroma and numerous fibroblasts with a distinct oval-shaped nucleus and abundant basophilic cytoplasm

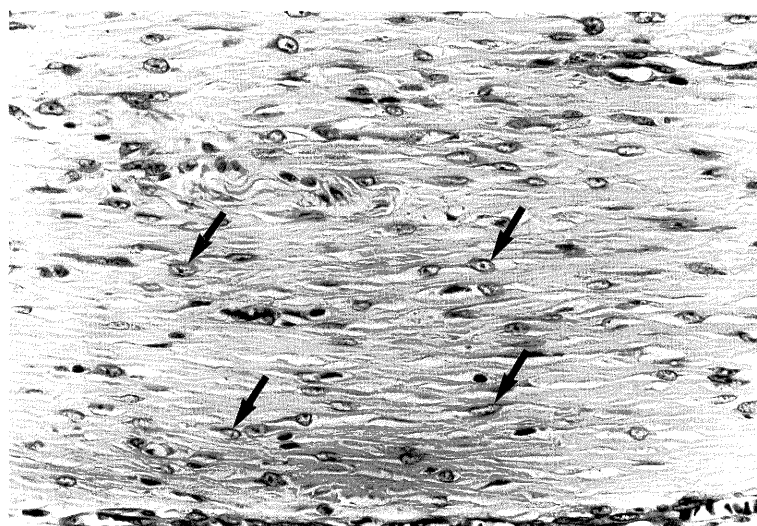


Fig. 4. F2-fibrosis. Intermediate features of the fibrosis between F1- and F3-fibrosis. Arrows indicate fibroblasts with an oval-shaped nucleus and indistinct cytoplasm. Hematoxylin and eosin stain. $\times 80$

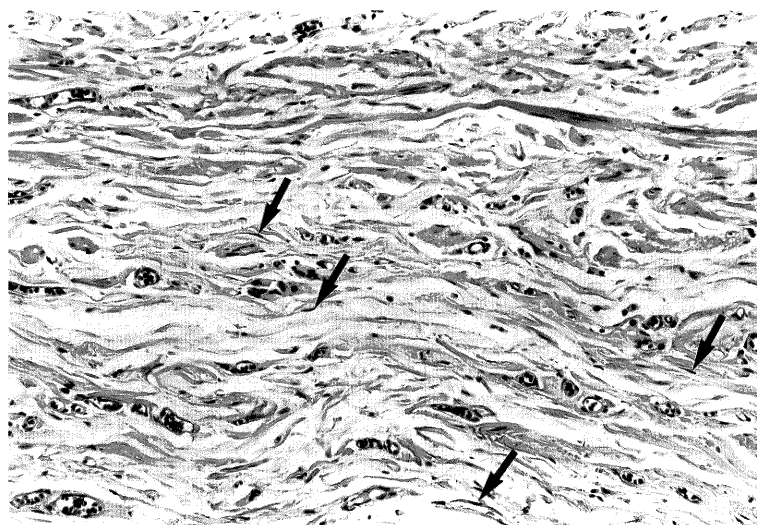


Fig. 5. F3-fibrosis. Note the loose collagen fibers and the sparse distribution of inactive fibroblasts (arrows). Hematoxylin and eosin stain. $\times 40$

(Fig. 3); F3-fibrosis characterized by loose collagen fibers and sparse distribution of fibroblasts with a spindle-shaped nucleus and inconspicuous cytoplasm (Fig. 5); and F2-fibrosis showing varying appearances of collagen fibers and fibroblasts. The most typical appearance of F2-fibrosis was, however, the presence of tight collagen fibers and fibroblasts characterized by an oval-shaped nucleus and indistinct cytoplasm (Fig. 4). F1-fibrosis was found in 7, F2-fibrosis in 16, and F3-fibrosis in 8 of 31 cases of ulcers or scars.

The ages of the ulcers surrounded by F1-, F2-, and F3-fibrosis are shown in Table 2. All of the ulcers surrounded by F1 fibrosis had an ulcer age of 0.8 months or less. F2-fibrosis appeared from 0.3 to 11.9 months after the onset of the ulcer. F3-fibrosis was present at 7 months or later. All but one (85.7%) of the 7 ulcers surrounded by F1-fibrosis were an open ulcer, eleven (68.8%) of the 16 ulcers having F2-fibrosis remained open, and all of the 8 ulcers associated with F3-fibrosis were a healed ulcer.

3. Correlation between the histologic changes of the regenerative mucosa and those of the submucosal fibrosis

As shown in Table 3, the histologic changes of the submucosal fibrosis correlated well with those of the regenerative mucosa according to the progress of ulcer healing. As the regenerative epithelium matur-

ated, F1-fibrosis was replaced by F2-fibrosis. Finally, F2-fibrosis changed into F3-fibrosis.

4. Fusion of the muscular layers

Fusion of the muscularis mucosae with the muscularis propria was observed in 10 (32.3%) of the 31 cases of ulcers or scars. The muscularis propria had been destroyed partially or completely by ulceration in all lesions with the fusion. The fusion appeared at 1.2 months or later and was present in all of the ulcers with the destruction of muscularis propria at 5.4 months or later.

DISCUSSION

Peptic ulcers of the stomach greatly vary in shape because ulcers tend to recur adjacent to the scar of the initial ulcer.⁹⁾ It has been reported that a round- or oval-shaped ulcer of the stomach implies an initial occurrence, while a linear or complex-shaped ulcer or scar develops as a result of repeated ulceration.¹⁰⁾ Histologic features at various healing stages of ulcers may simultaneously be present in a recurrent ulcer, which may evoke serious confusion in histologically evaluating the age of an ulcer. Therefore, the discrimination between an initial and a recurrent ulcer is essential for a chronological assessment of histologic changes of gastric ulcers. In the present study, we selected stomachs having round- or oval-shaped ulcers or scars for these reasons.

In agreement with previous reports,³⁻⁶⁾ regenerative epithelium extended toward the center of the ulcer base in a membranous or papillary appearance at the earliest stage of ulcer healing (immature stage); subsequently the regenerative tissue increased in height, showing a tufty configuration with a few pseudopyloric glands newly formed (juvenile stage); finally it became as mature as the intact mucosa of the stomach (mature stage). As demonstrated in the present study, histologic features of the regenerated mucosa can be considered one histologic marker indicating ulcer age. Our results concerning the time required for the maturation of the regenerative epithelium are consistent with those reported by Kubo.⁶⁾ Interestingly, regeneration of parietal cells and chief cells was observed in some lesions of our series, although Helpap et al.¹¹⁾ failed to confirm the regeneration of these cells in the healing of an experimental ulcer in rats. This failure on their part may be ascribed to the relatively short period of their observation, as parietal cells in our series appeared in the

Table 2. Histologic features of the submucosal fibrosis in relation to the age of gastric ulcer

Fibrosis	n	Ulcer age (months)	(median)
F 1	7	0.1- 0.8	(0.5)
F 2	16	0.3-11.9	(1.5)
F 3	8	7 -48	(10.5)

Table 3. Relationship between the histologic features of the regenerative epithelium and those of the submucosal fibrosis according to the progress of healing in gastric ulcer

Regenerative epithelium	n	Submucosal fibrosis		
		F1 (%)	F2 (%)	F3 (%)
None*	2	2 (100)	0 (0)	0 (0)
Immature stage	10	5 (50)	5 (50)	0 (0)
Juvenile stage	8	0 (0)	8 (100)	0 (0)
Mature stage	11	0 (0)	3 (27.3)	8 (72.7)

*no emergence of the regenerative epithelium at the ulcer margin

regenerated mucosa at 77 days or later, and chief cells, at 315 days after the ulceration. Moreover, it is of particular interest that intestinal metaplasia developed in the regenerative mucosa in two cases of the present series, supporting the previously reported hypothesis that intestinal metaplasia develops in the regenerative changes of the gastric mucosa.^{12,13)}

In addition to the histologic features of the regenerative mucosa, those of the submucosal fibrosis surrounding an ulcer are considered a histologic indicator of ulcer's age. F1-, F2-, and F3-fibrosis approximately indicate the ulcer age of one month or shorter, one to 12 months, and one year or longer, respectively. As far as we have investigated, this is the first report on the significance of the histologic features of the submucosal fibrosis evoked by the gastric ulcer in evaluating the age of the ulcer. Furthermore, the fusion of muscularis mucosae with muscularis propria is regarded as another histologic marker of the ulcer's age, although its application is confined to ulcers with destruction of the muscularis propria. Myofibroblasts which proliferate in the submucosa after the initiation of ulcer as reported by Nakamura et al.⁷⁾ may play an important role in causing the fusion of the two muscular layers of the stomach, although the mechanism of the fusion has not been elucidated yet. In our series, the fusion developed at 36 days or later, which is consistent with the results of Kubo.⁶⁾

Studies have shown that peptic ulcer occasionally develops inside an early gastric cancer and shows the same course of healing as that of a benign gastric ulcer (malignant cycle).¹⁴⁾ It seems to be difficult to estimate the chronology of a malignant ulcer by the histologic appearances of the regenerative mucosa because it is most likely infiltrated by cancerous tissue. Even in such cases, histologic features of the submucosal fibrosis around the ulcer and, if present, the fusion of the two muscular layers may be of use in evaluating the age of the malignant ulcer.

In conclusion, histologic features of the regenerative mucosa covering the ulcer base, those of the submucosal fibrosis surrounding the ulcer, and the presence or absence of the fusion of the lamina muscularis mucosae with the tunica muscularis propria are considered reliable histologic indicators of the age of a gastric ulcer.

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