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An Easy Method to Identify Parietal Cells in Gastric Biopsies

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Abstract. *Background: Sections from gastric biopsies are usually stained with hematoxylin and eosin (H&E), a stain that is not optimal for the recognition of many parietal cells. This paper describes a more suitable routine stain to identify parietal cells. Patients and Methods: Nineteen sets of gastric biopsies were consecutively stained with H&E and with modified Giemsa. Giemsa-stained fundic biopsies showed a parietal cell band intercalated between the fovelar epithelium and the chief glands. The continuity of this band was studied at ×4 magnification and its thickness in one well-oriented field at ×10 magnification. Results: A distinct, continuous parietal cell band was recorded in fundic biopsies exhibiting normal mucosa, acute gastritis or chronic gastritis without glandular atrophy (Group A). A discontinuous or lack of parietal cell band was found in fundic biopsies exhibiting chronic gastritis with glandular atrophy or with intestinal metaplasia (Group B). The ratio of parietal cell band/total mucosal thickness ranged between 0.30 and 0.40 in Group A and between 0 and 0.25 in Group B. Conclusion: A parietal cell band was readily demonstrated in sections from gastric biopsies stained with Giemsa, but not in those stained with H&E. Discontinuity with reduced or absent band was recorded in gastric diseases characterised by a decrease of the parietal cell population.*

The stomach is classically subdivided into 3 different histological mucosal zones: i) cardia mucosa, ii) fundic or oxyntic mucosa and iii) pyloric-antrum mucosa, each one with its characteristic phenotype (1).

In recent years, the identity of the cardia mucosa has been questioned, the claims being that under the influence of protracted gastric reflux, the squamous mucosa of the distal oesophagus undergoes glandular metaplastic transformation (2, 3).

The fundic mucosa is composed of mucus secreting foveolar cells, of hydrochloric acid (HCA) and intrinsic factor producing parietal cells and of pepsin secreting chief cells. The pyloric-antrum mucosa is built of mucus-secreting foveolar cells and of mucus- and gastrin-producing pyloric glands (1). At the *incisura angularis* the transitional mucosa is either of fundic type, of pyloric-antrum type, or of an admixture of both (4). Gastrin stimulates parietal cells to produce HCA by binding CCK2/gastrin receptors on endocrine-like cells (ECL) through the release of histamine (5). Parietal cells secrete H^+ ions in a paracrine manner. Gastrin also causes chief cells to secrete pepsinogen, which is converted into pepsin under the influence of HCA production and of pancreatic enzymes from acinar cells (6).

The most common diseases affecting the gastric mucosa are acute gastritis and chronic gastritis with or without active inflammation often evoked by *Helicobacter pylori* infection, sequels of gastritis such as mucosal atrophy, intestinal metaplasia and pseudopyloric metaplasia (7) and mucosal tumours. A less common malady is autoimmune gastritis (8), which causes the destruction of the parietal cells leading to achlorydria, deficiency in intrinsic factor, vitamin B12 absorption and megaloblastic anaemia. Most of these diseases affect the bulk of the parietal cell population.

In hematoxylin and eosin (H&E)-stained sections, parietal cells are depicted as triangular in shape with a centrally located nucleus and an eosinophilic cytoplasm (1). The discrimination between parietal cells and chief cells in H&E -stained sections, is, however, not straightforward. Hence, one plausible cause for the difficulties in detecting many of these cells could be that H&E is not an ideal stain for the visualization of all parietal cells. Gastric biopsies showing an unquestionable reduction of parietal cells in the fundic mucosa (as deduced by the lower number of glands/field often replaced by inflammatory cells) are consistent with atrophic gastritis (7). However, early stages of atrophic gastritis might be more difficult to recognize. Hence, a method that would permit the easy identification of parietal cells in conventionally stained gastric biopsies is desirable.

In daily praxis, Giemsa stain is used to detect *Helicobacter pylori* in gastric biopsies (7). Searching for *H. pylori* infection in Giemsa-stained sections, it became apparent that parietal cells could easily be identified. The purpose of this work was to report this novel method of parietal cell recognition in a cohort of clinical gastric biopsies.

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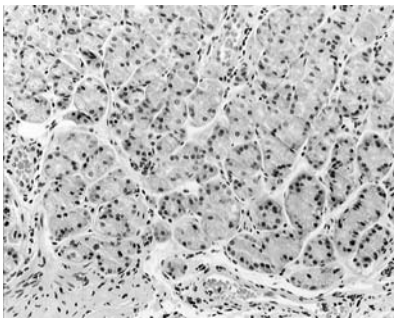

Figure 1. *Normal fundic mucosa. Parietal cells are difficult to differentiate from chief cells. H&E stain (×20 magnification).*

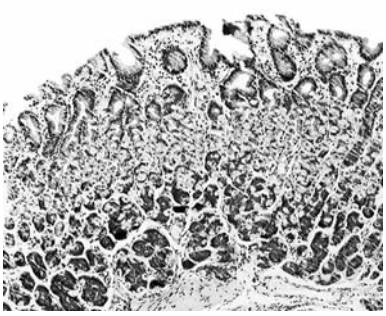

Figure 2. *Normal fundic mucosa. Note the presence of a parietal cell band intercalated between the darker foveolar-neck epithelia on top and the chief cell glands at the bottom. Modified Giemsa stain (×10 magnification).*

Patients and Methods

Nineteen consecutive sets of biopsies containing gastric mucosa from the fundus and from the pyloric-antrum were investigated. Cases (11 males and 8 females) were selected when at least one of the following prerequisites were fulfilled: normal gastric mucosa (n=7), acute (active) inflammation (n=3), chronic inflammation without glandular atrophy (n=3), chronic gastric inflammation with mild/moderate glandular atrophy of the fundic mucosa (n=3), or intestinal metaplasia in antrum and fundic mucosa (n=3).

Sections were alternatively stained with H&E (Figure 1) and with a modified Giemsa stain (9, 10) (Figure 2) and inspected using a conventional microscope (Labophot-2, Nikon, Japan). Only well-oriented mucosal fields were included in the study.

A distinct parietal cell band (defined as a transversal field of parietal cells intercalated between the fovelar epithelium and the chief glands) was seen in Giemsa-stained sections (Figure 2). Parietal cells were slightly stained, thus contrasting against the darker stained foveolar, neck and chief cells.

The continuity of this band was assessed at ×4 magnification and its thickness using an ocular microscale in one well-oriented field at ×10 magnification. The ratio of parietal cell band/total mucosal thickness was then calculated. Occasional parietal cells were also found outside the parietal cell band, amidst the neck cells and the chief cells. These cells were not included in calculating the ratio of parietal cell band/total mucosal thickness. No to occasional parietal cells were found in Giemsa-stained sections from the pyloric-antrum in the 19 cases.

Results

The continuity as well as the ratio of the thickness of the parietal cell band to the whole thickness of the fundic mucosa in 19 gastric biopsies from the corpus is shown in Table I. A distinct, continuous parietal cell band was recorded in fundic biopsies exhibiting normal mucosa, acute gastritis or chronic gastritis without glandular atrophy (Group A). A discontinuous or absent parietal cell band was found in fundic biopsies exhibiting chronic gastritis with glandular atrophy (Figure 3) or with intestinal metaplasia (Figure 4) in Group B. The ratio of parietal cell band/total mucosal thickness ranged between 0.30 and 0.40 in Group A and between 0 and 0.25 in Group B.

Discussion

Gherardi *et al*. (11) tested four different stains to study parietal cells in the rat stomach: i) a modification of the H&E method proposed by Drysdale and Marks (12), ii) hematoxylin-eosin-saffron fluorochrome stain, iii) hematoxylin-azophloxin-saffron fluorochrome stain, and iv) May-Grunwald-Giemsa stain on thin sections from plastic-embedded specimens. The May-Grunwald-Giemsa stain using plastic-embedded specimens provided the best results for the visualization of parietal cell in rat stomachs (11).

To study gastric parietal cells, other methods have been proposed such as WGA and HPA-L lectins in guineapig stomachs (13), flow-cytometry in mouse and guineapig stomachs (14, 15) and in human gastric biopsies (16), huntington interacting protein 1 in mice stomachs (17), parietal-specific H^+K^+-ATPase antibody in mase stomachs (18, 19), as well as prostaglandin E_2 (20) and transmission electron microscopy in stomachs of laboratory animals (21, 22). These methods are laborious, costly, and time-consuming, and to date, none of them have been applied to retrospectively or prospectively investigate possible alterations in the parietal cell population in human gastric biopsies in health and disease.

In present study, H&E stain proved to be unreliable in identifying many parietal cells, as the parietal cell band, clearly detected using Giemsa stain, remained undetected using H&E stain. The limitation in recognizing many parietal cells with H&E stain became apparent when three male pathologists attempted to identify parietal cells in a multi-headed microscope. Although the cause for this difficulty remains unclear, it seems to be unrelated to the fact that 10% of male pathologists (a sample from the general population)

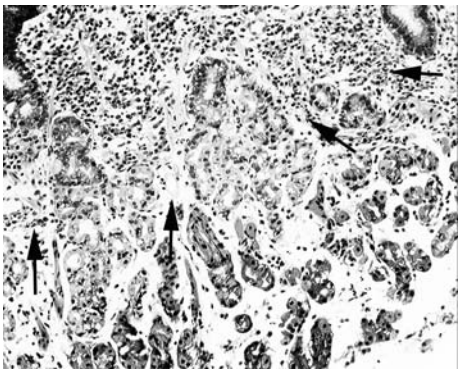

Figure 3. *Fundic mucosa with chronic inflammation. Note the disruption of the parietal cell band at arrows. Modified Giemsa stain (×20 magnification).*

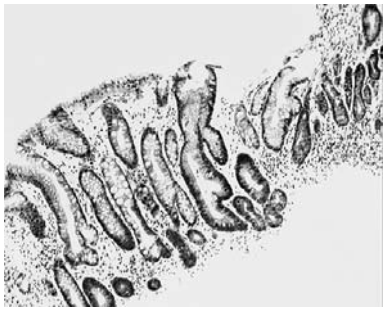

Figure 4. *Fundic mucosa with intestinal metaplasia. Note the absence of the parietal cell band. Modified Giemsa stain (×10 magnification).*

Table I. *Continuity versus discontinuity of a parietal cell band, as well as the ratio parietal cell band/total thickness of the fundic mucosa, in sections from 19 gastric biopsies stained with Giemsa.*

Histological diagnosis/cases	Continuity *versus* discontinuity of the parietal cell band	Ratio parietal cell band/ total mucosal thickness
Normal	Continuous	2/5 (0.40)
Normal	Continuous	2/6 (0.33)
Normal	Continuous	2/5 (0.40)
Normal	Continuous	2/5 (0.40)
Normal	Continuous	2/5 (0.40)
Normal	Continuous	2/6 (0.33)
Normal	Continuous	2/5 (0.40)
Normal	Continuous	1.5/5 (0.30)
Normal	Continuous	2/6 (0.33)
Acute gastritis*	Continuous	2/6 (0.33)
Acute gastritis*	Continuous	1.5/5 (0.30)
Acute gastritis*	Continuous	2/5 (0.40)
Atrophic gastritis (mild)	Discontinuous	1/4 (0.25)
Atrophic gastritis (moderate)	Discontinuous	0.5/5 (0.10)
Atrophic gastritis (moderate)	Discontinuous	1/5 (0.20)
Intestinal metaplasia	Discontinuous	0.5/4 (0.13)
Intestinal metaplasia	Discontinuous	0.2//4 (0.05)
Intestinal metaplasia	Absent	0/4 (0)

*No glandular atrophy.

suffer from Daltonism (23) a drawback when male pathologists try to detect cells with eosinophilic cytoplasm. In this context, the review of H&E-stained gastric sections from a transgenic mouse model performed by the (male) author and a female pathologist (24) revealed that only some parietal cells could be recognised with certainty by the two observers, even in stomachs from wild animals having normal fundic mucosa.

Based on this experience, five H&E sections from patients having normal fundic mucosa were reviewed in a multi-headed microscope, together with two female pathologists working at this laboratory. The female pathologists (who were unaware of the purpose of the review) were asked to identify all parietal cells. Here again, only some parietal cells were discriminated with certainty. In contrast, all three pathologists (two females and one male) agreed upon the presence of a parietal cell band in Giemsa-stained sections. In addition, other parietal cells present amidst the neck cells and the chief cells were easily identified with this stain (unpublished observations). Hence, the gender of the observer was not the main reason for the difficulty in recognising many parietal cells in H&E-stained sections. H&E stain was found to be not ideal for recognizing many parietal cells in sections from gastric biopsies.

In conclusion, discontinuity of the parietal cell band, reduced amplitude or absence of this band was recorded in Giemsa-stained fundic sections from gastric diseases known to have a decreased parietal cell population.

It should be noted that another advantage of using modified Giemsa to stain fundic biopsies is that male pathologists with Daltonism (23) may benefit from this method, as parietal cells are easily recognised by their pale staining in Giemsa stain and not by the eosinophilic cytoplasm in H&E stain.

Modified Giemsa stain has been used at this laboratory to diagnose *Helicobacter pylori* infection in gastric biopsies since 1992 (7). Accordingly, modified Giemsa-stained archival sections available in our files will be reviewed, aiming to investigate the amplitude of the parietal cell band in a larger cohort of biopsies from patients with various disorders characterised by a reduction of the parietal cell population.

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