

# A simple method to demonstrate duodenal gastric metaplasia

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**Aims:** The diagnosis of duodenal gastric metaplasia (DGM) is based on the demonstration of periodic acid Schiff (PAS) positive mucin in duodenal columnar cells. Recently, groups of duodenal columnar cells were seen to be autofluorescent in haematoxylin and eosin (H&E) stained sections from a patient with DGM.

**Materials and methods:** Consecutive archival gastric and duodenal H&E sections from 30 patients with chronic gastritis and DGM (CG+DGM), from 30 with chronic gastritis without DGM (control group I), from 30 with normal gastric and duodenal mucosa (control group II), and from five patients with coeliac disease (control group III) were reviewed on a fluorescent microscope.

**Results:** The surface epithelium of the gastric mucosa in all 95 cases had autofluorescent material. In 31 cases with DGM (including one unreported case in control group I), groups of columnar duodenal cells also had autofluorescent material. In the remaining 64 cases, duodenal columnar cells were not autofluorescent. Results were confirmed with the PAS stain.

**Conclusions:** The method described detected apical mucin secretion in columnar cells, both in the stomach and in the duodenum from patients with DGM. The autofluorescence was induced by eosin (which binds to neutral mucin). Observing H&E stained duodenal biopsies under a fluorescence microscope may be sufficient to confirm DGM or to detect incipient DGM. Despite long observation periods and a long exposure time while photographing, the autofluorescence did not fade away. Because re-cuttings for special staining (PAS) are no longer required when this method is used, both final diagnosing time and laboratory costs can be reduced.

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Duodenal gastric metaplasia (DGM) is a histologically detectable mucosal injury caused by abnormally large quantities of gastric acid reaching the duodenal cap.<sup>1</sup> DGM leads to peptic duodenitis. The hyperproduction of gastric acid usually results from infection of the gastric mucosa with *Helicobacter pylori*.<sup>2</sup> DGM can also be found in the absence of *H pylori* infection—for example, in Crohn's disease of the duodenum, and less frequently in coeliac disease.<sup>1,2</sup>

In the normal stomach, the columnar cells of the surface epithelium secrete mucins that can be stained with periodic acid Schiff (PAS).<sup>3</sup> In the normal duodenum, the villi are lined with columnar cells and with goblet cells. Columnar cells have a striated absorptive border and do not secrete mucin, whereas goblet cells secrete mainly sialomucins, which are stained with PAS and more specifically with the cationic dye alcian blue (AB).<sup>3</sup>

In DGM, the surface columnar cells resemble the normal superficial columnar cells of the gastric mucosa in that they acquire the capacity to secrete mucin.<sup>1,2</sup>

"The hyperproduction of gastric acid usually results from infection of the gastric mucosa with *Helicobacter pylori*"

Recently, while investigating archival haematoxylin and eosin (H&E) sections from the duodenal mucosa—with the purpose of analysing the characteristics of the basement membrane using a fluorescent microscope<sup>4</sup>—we found autofluorescent material in groups of superficial columnar duodenal cells. This autofluorescent material was PAS positive and the case was diagnosed as DGM.

The purpose of our present study was to investigate, in consecutive duodenal biopsies from patients previously diagnosed with DGM, whether the pathological mucin contained in duodenal columnar cells was autofluorescent.

## MATERIAL AND METHODS

Consecutive archival H&E stained sections from the duodenum of 30 patients with chronic gastritis and DGM (CG+DGM), 30 patients with normal gastric and duodenal mucosa (control group I), 30 patients with chronic gastritis without DGM (control group II), and five patients with coeliac disease and duodenal villous atrophy (DVA, control group III) were reviewed under an Axioskop routine microscope (Carl Zeiss, Wetzlar, Germany) using alternatively transmitted light and incident light fluorescence (green H filter, 546 nm wl).

Giemsa stain was available in all cases. Additional sections were stained with AB and high iron diamine to label sialomucins and sulfomucins, respectively.<sup>3,5,6</sup> When those stains were negative, a positive PAS stain was considered to be evidence of the presence of neutral mucins.<sup>3</sup>

DGM was defined as the occurrence of foci of duodenal cells without a brush border, containing apical mucous.

The Student's unpaired *t* test was used to compare the significance of the difference between the group means. Significance was set at  $p < 0.05$ .

## RESULTS

### Patients

In the chronic gastritis and DGM group (CG+DGM), there were 18 male and 12 female patients. In control group I, there were 16 male and 14 female patients, and in control group II there were 17 male and 13 female patients. The mean (SD) age in the 30 patients with chronic gastritis and DGM (CG+DGM)

**Abbreviations:** AB, alcian blue; CG, chronic gastritis; DGM, duodenal gastric metaplasia; DVA, duodenal villous atrophy; H&E, haematoxylin and eosin; PAS, periodic acid Schiff

**Table 1** The number of patients showing autofluorescent material in haematoxylin and eosin stained duodenal sections excited with incident light fluorescence

	No. of patients	Superficial gastric cells	Columnar duodenal cells with DGM
CG+DGM	30	30/30	30/30
Control group I	30	30/30	0/30
Control group II	30	30/30	1/30
Control group III	5	5/5	0/5
All	95	95/95	31/95

CG+DGM, patients with chronic gastritis (GC) and duodenal gastric metaplasia (DGM). Control group I, patients with normal gastric and duodenal mucosa at initial diagnosis. Control group II, patients with chronic gastritis without duodenal gastric metaplasia at initial diagnosis. Control group III, patients with duodenal villous atrophy at initial diagnosis.

was 48 (10) years, in group I it was 44 (15) years, and in group II it was 45 (12) years. No significant differences in sex and age were found between these three groups.

The mean age in the five patients with DVA was 16.6 years (range, 2–71 years).

### Histology

Table 1 shows that in the gastric mucosa the apical mucous of the superficial foveolar epithelium was autofluorescent in all 95 patients investigated (fig 1).

In the duodenal mucosa, the cytoplasm of the columnar and goblet cells in all 30 patients from control group I (with normal duodenal mucosa) and in 29 of the 30 patients from control group II (with chronic gastritis without DGM) were non-fluorescent (fig 2). However, the brush border of the columnar cells was autofluorescent.

At re-evaluation, the remaining case in group II (initially reported as normal on the basis of H&E stained sections alone) showed groups of columnar cells having autofluorescent material in their cytoplasm. That material was PAS positive.

Similar to the superficial epithelium of the gastric mucosa, all 30 patients with CG+DGM had groups of columnar super-

ficial duodenal cells harbouring apical autofluorescent material (fig 2C). The cytoplasm in the remaining superficial cells of the duodenum, in addition to the goblet cells, was not fluorescent ( $n = 30$ ).

Traces of sialomucins (as deduced by a weak AB stain) were detected in some cells with DGM. The HID stain was negative.

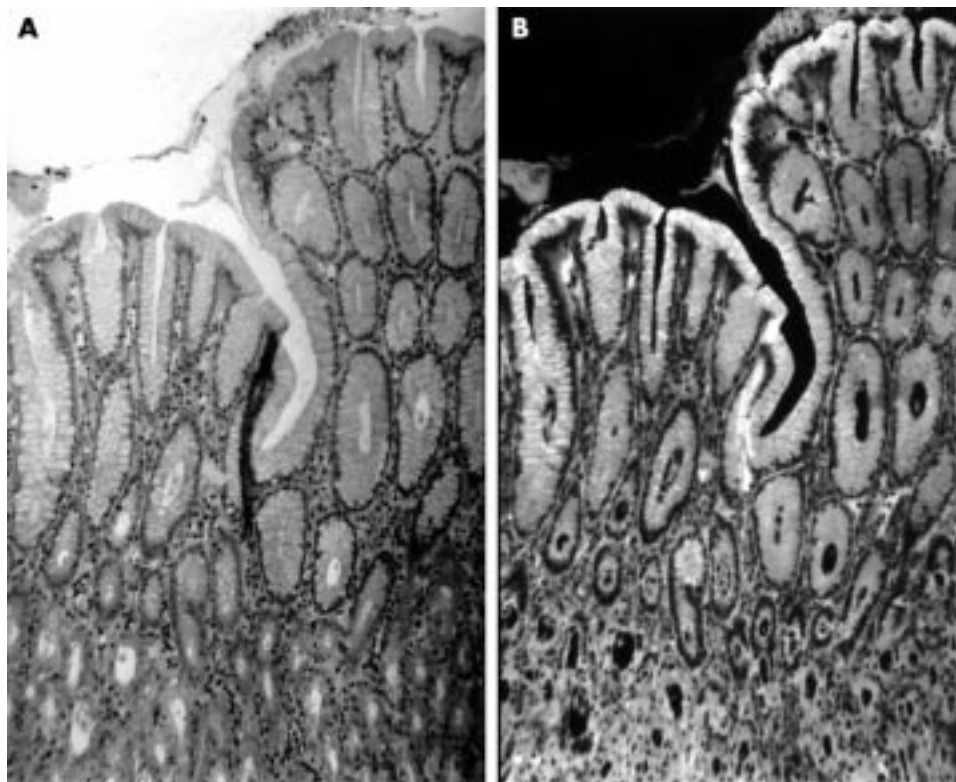
All 30 patients with CG+DGM harboured *H pylori* in their gastric specimens, as did 18 of 30 patients with chronic gastritis without DGM. None of the normal patients harboured *H pylori*.

In all five patients from control group III (with DVA), the cytoplasm of the columnar and goblet cells of the duodenal mucosa was not fluorescent.

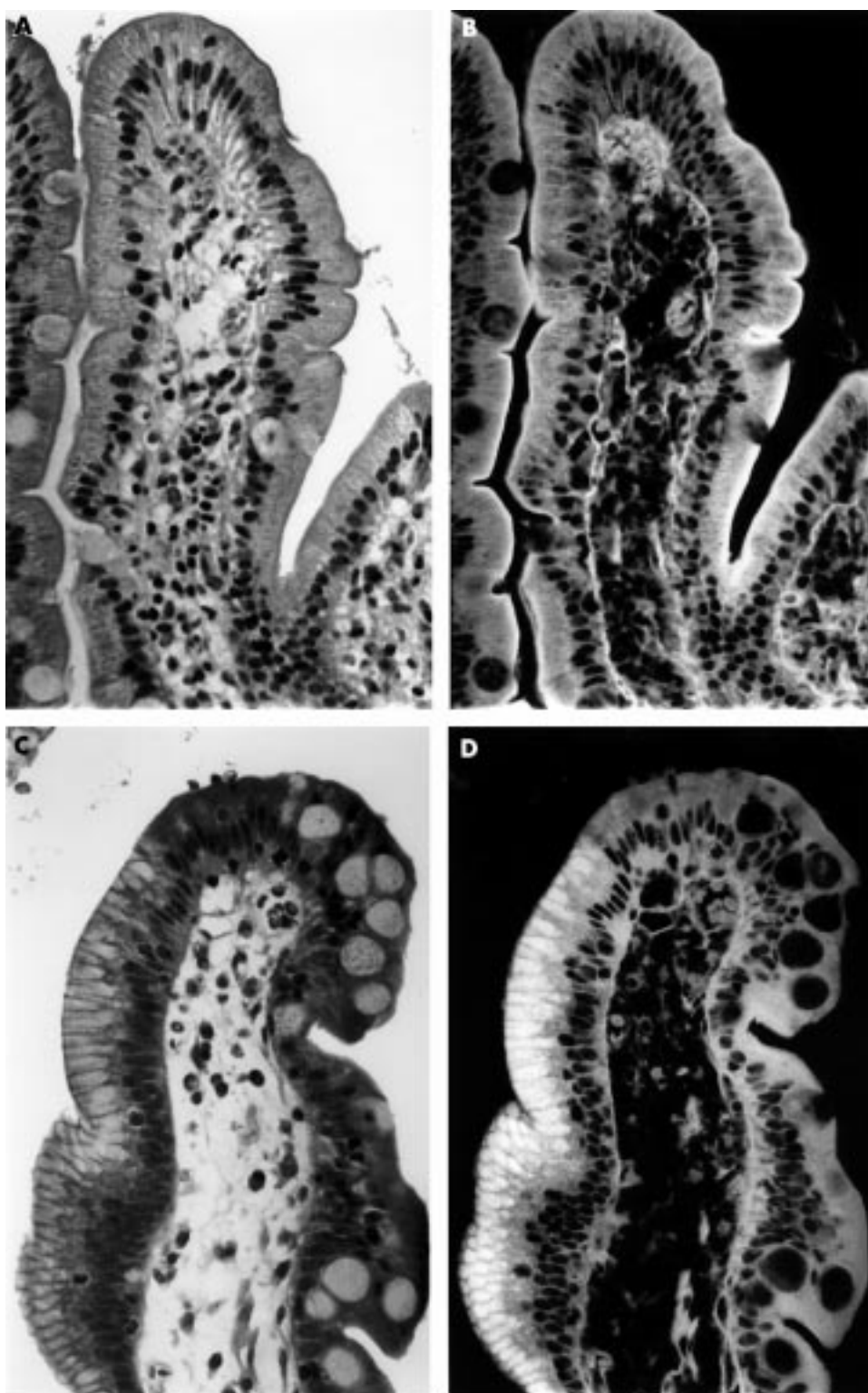
### DISCUSSION

In the normal stomach, the luminal aspect of the cytoplasm of the surface and foveolar columnar cells contains neutral mucin. In the neck region of the glands of the body of the stomach, traces of acid mucin may be present, but only in small amounts.<sup>3</sup>

In the normal duodenum, the jejunum, and the ileum at the luminal aspect of the cytoplasm of the surface columnar cells



**Figure 1** (A) Haematoxylin and eosin (H&E) stained section from normal gastric mucosa as seen with transmitted light (original magnification,  $\times 25$ ). (B) The same area as (A) observed with incident light fluorescence. Note autofluorescent material in the superficial epithelium (H&E; original magnification,  $\times 25$ ).



**Figure 2** (A) Haematoxylin and eosin (H&E) stained section from normal duodenal mucosa as seen with transmitted light (original magnification,  $\times 100$ ). (B) The same area as (A) observed with incident light fluorescence. Only the brush border and the basement membrane are autofluorescent (H&E; original magnification,  $\times 100$ ). (C) H&E stained section from duodenal mucosa with duodenal gastric metaplasia, as seen with transmitted light (original magnification,  $\times 100$ ). Note apical vacuolisation of the columnar cells on the left slope of the villi. (D) The same area as (C) when observed with incident light fluorescence. Note that the material contained in the apical vacuolisation of the columnar cells shown in (C) is autofluorescent (H&E; original magnification,  $\times 100$ ).

does not contain neutral mucin, sialomucins,<sup>1</sup> or sulfomucins.<sup>3</sup> In contrast, the cytoplasm of columnar cells of the duodenum with DGM contains PAS positive mucin and traces of AB positive sialomucins. Consequently, most of the PAS positivity in DGM cells is the result of neutral mucin,<sup>3</sup> because neutral mucin is defined by the failure to demonstrate acid groups reacting with cationic dyes (AB or HID).<sup>3</sup>

In our present study, autofluorescent material was demonstrated in H&E stained sections excited with fluorescent light. That material was present not only in the cytoplasm of the superficial foveolar columnar cells of the stomach in all three groups investigated, but also in the cytoplasm of superficial columnar duodenal cells from patients with DGM. The autofluorescent material was PAS positive. Obviously,

autofluorescence demonstrated those cells able to secrete neutral mucin (as deduced by histochemistry).

The presence of neutral mucins in the cytoplasm of DGM cells seems to be the result of a cellular adaptation to the chronic insult caused by the noxious gastric juices acting upon the duodenal mucosa.<sup>3</sup> Because neutral mucins are normally secreted by the superficial foveolar columnar cells of the gastric mucosa, it may be inferred that neutral mucin exerts a substantial buffer effect upon gastric acids, not only in the gastric milieu but also in the microenvironment surrounding DGM cells.

The reason for the absence of DGM in 29 of the 30 patients in control group II remains unclear. It is conceivable that the development of DGM may be influenced by the duration of



infection with *H pylori*, by the presence of gastric hypochlorhydria or achlorhydria, by antacid/anti-*H pylori* medication, and/or by the sensitivity of the patient to the harmful HCl acting upon the duodenal mucosa.

"The autofluorescence of the eosin did not fade away despite long periods of observation under the fluorescent microscope and long periods of exposure time while photographing"

We investigated the possible cause(s) of the autofluorescence seen in the apical mucous of superficial columnar cells. For this purpose, duodenal sections from 12 patients were stained exclusively with eosin (Polysciences Incorporated, Warrington, Pennsylvania, USA), exclusively with haematoxylin (Gill's haematoxylin III; Merck, Darmstadt, Germany), or exclusively with PAS. One set of sections was not stained. Results showed that only sections stained exclusively with eosin displayed autofluorescence of the apical mucous (both in the superficial cells of the normal stomach and in the DGM cells of the duodenum). Sections that had been stained with all the other stains, in addition to the unstained sections, remained non-fluorescent. Thus, the autofluorescence reported here was exclusively elicited by the eosin of the H&E stain.

The autofluorescence of the eosin did not fade away despite long periods of observation under the fluorescent microscope and long periods of exposure time while photographing. This was very useful because it permitted repeated inspections of archival H&E stained sections under the fluorescent microscope, in the search for undiagnosed cases of DGM.

From our data, we suggest that the simple observation of H&E stained duodenal biopsies under a fluorescent microscope may be sufficient to confirm DGM. Equivocal cases of DGM (using light microscopy) may be easily confirmed or rejected by scrutinising H&E stained sections under a fluorescent microscope. Using this simple procedure, re-cuttings for special staining are no longer required, and consequently, the

### Take home messages

- This method can detect apical mucin secretion in columnar cells, both in the stomach and in the duodenum, in haematoxylin and eosin stained slides from patients with duodenal gastric metaplasia (DGM)
- The method could be useful in the diagnosis of equivocal cases of DGM
- The autofluorescence was induced by eosin (which binds to neutral mucin)
- Despite long observation periods and a long exposure time while photographing, the autofluorescence did not fade away
- This method could help reduce both the time to final diagnosis and laboratory costs

final diagnosing time and laboratory costs have been reduced in our department.

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## ECHO

### Active ingredient



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Tests in patients with colorectal disease have confirmed that lipocalin—a protein released from neutrophils—is a specific marker for monitoring neutrophil activity in the inflammatory process. Demonstrating the secretion and specificity of human neutrophil lipocalin (HNL) in vivo confirms the researchers' previous work on isolation of HNL from the secondary granules of neutrophils and makes HNL ideal for studying neutrophil behaviour in the local inflammatory process characteristic of ulcerative colitis.

Perfusion tests showed much higher amounts of HNL in the perfusion fluid from rectal and sigmoid segments of the intestine in patients with proctitis (median concentration 245 µg/ml (upper-lower quartile 149–557 µg/ml; 13.4 µg/ml (7.8–21.4 µg/ml) respectively) and colitis (358 µg/ml (55.2–430 µg/ml); 105 µg/ml (19.6–586 µg/ml) respectively) than tests in healthy controls (rectal 9.2 µg/ml (3.3–13.2 µg/ml); sigmoid (<2 µg/ml)). HNL was shown histochemically in mucosal specimens from these patients to be confined exclusively to neutrophils—in the lamina propria, between crypt cells, and in the lumen of crypts in inflamed areas of the rectal and sigmoid intestine. The amount of HNL in the perfusion fluid correlated significantly with clinical scores for disease for rectal segments in patients with proctitis and both rectal and sigmoidal segments for colitis.

Eighteen patients—10 with colitis and eight with proctitis—and 18 healthy controls were perfused in the sigmoid and rectal segments of their intestine. HNL released into the fluid was subsequently measured by radioimmunoassay. Mucosal tissue from the perfused segments was obtained by biopsy in all subjects, and thin sections were stained immunohistochemically with a mixture of six monoclonal antibodies specific for HNL.

▲ *Gut* 2002;**50**:501–506.