

Quantification of human gastric G cell density in endoscopic biopsy specimens: effect of shape of specimen

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SUMMARY Experiments performed on endoscopic biopsy specimens show that when they are curved, as they often are, the inner surface of the mucosa is contracted and the outer surface is expanded. When estimating G cell numbers per unit length of mucosa from such biopsy specimens, artefacts produced by variations in shape are minimised by relating the number of G cells to the mean of the length of the muscularis mucosae and the length of the outer mucosal profile on the tissue section containing these cells.

Various methods of estimating the number of gastrin-containing cells in the stomach have been described. Some studies have been performed on gastrectomy specimens.^{1,2} Other workers have tackled the more difficult problem of using biopsy specimens taken at endoscopy,^{3,4} thus allowing studies to be performed on more than one occasion on the same patients and on subjects not undergoing gastric resection.

Although some workers have related the number of G cells to the volume of mucosa in which they lie,^{5,6} it has been pointed out by Piris and Whitehead⁴ that relating this number to the area occupied by the particular specimen of mucosa eliminates variation due to changes of mucosal thickness, such as those which occur in gastritis. This is particularly relevant when studying patients with peptic ulcer disease since gastritis is usually associated with gastric ulcer⁷ and is common in patients with duodenal ulcer.⁸ The number of G cells per unit length of a section of mucosa, henceforth referred to as the G cell density, is related to the number of G cells per unit area of mucosa represented by the section. Piris and Whitehead⁴ related the number of G cells to the length of the muscularis mucosae in the section. This was estimated by counting the number of times the line representing the mucosal surface of this structure intersected with lines on a test grid. They used the muscularis mucosae because they considered that this was the part of the mucosa most likely to retain its length and thus reflect the true length of the specimen.

One of the features of endoscopic biopsy specimens is that they vary in shape. When they are taken, they consist of a strip of mucosa folded in half with the mucosal surface on the outside. It is possible to unfold the fresh specimen and orientate it on a piece of filter paper for fixation, with the mucosal surface uppermost. The result of this will be either a flat specimen (shape A, Fig. 1) or a slightly curved one (shape B, Fig. 1), depending on the amount of submucosa included in the specimen. If the specimen is not unfolded before being placed in fixative, it may remain markedly curved (shape C, Fig. 1) or assume a more complex shape such as that of the fourth example shown in Figure 1.

The purpose of the present study was to determine which of the following best reflected the true length of the biopsied mucosa: the length of the muscularis mucosae, the length of the outer mucosal profile, or the mean of these two (Fig. 2). In other words, when the specimen is curved, has the inner surface shrunk, the outer surface expanded, or have both these processes occurred?

Material and methods

Biopsy specimens were obtained from patients undergoing upper gastrointestinal endoscopy with an Olympus GIF-K endoscope using the standard biopsy forceps (FB-3K) with 2 mm diameter hemispherical cups. Suitable specimens can be obtained reliably by allowing the stomach to contract a little. This increases the laxity of the mucosa, making it more likely to fold as the forceps are closed. When the stomach is distended with air, the

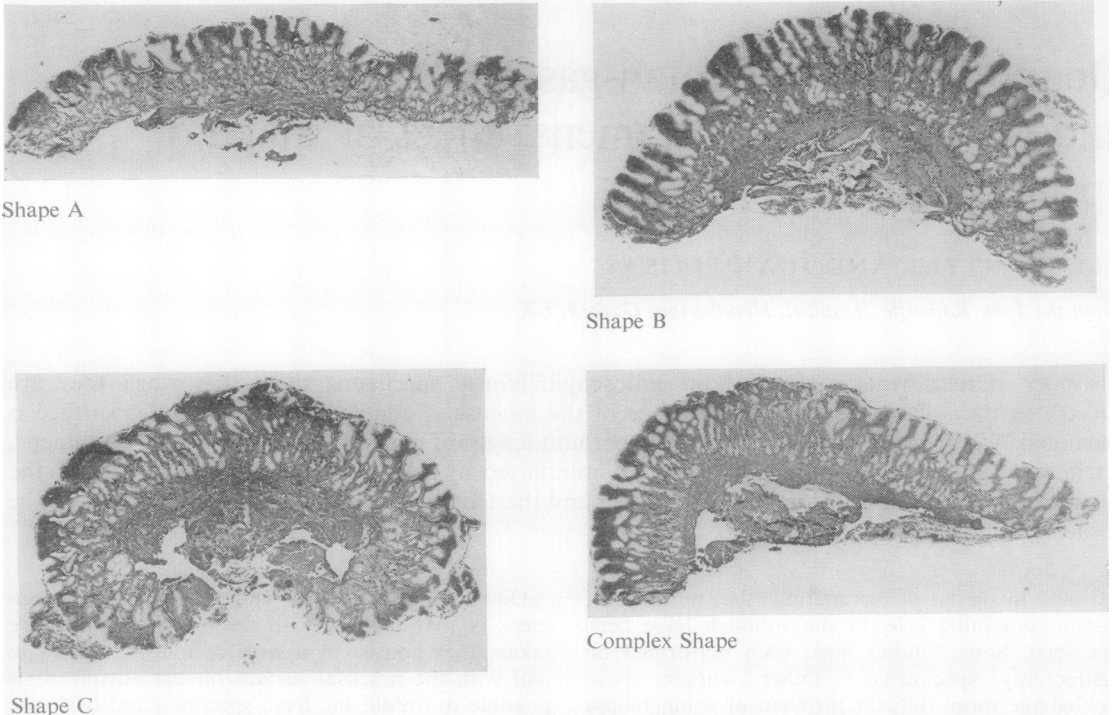


Fig. 1 Examples are shown of biopsy specimens of different shapes. Flat specimens were designated shape A. Slightly curved and markedly curved specimens were designated shape B and shape C respectively. The fourth example is of a specimen of complex shape in this instance consisting of a flat part and a curved part (A + B).

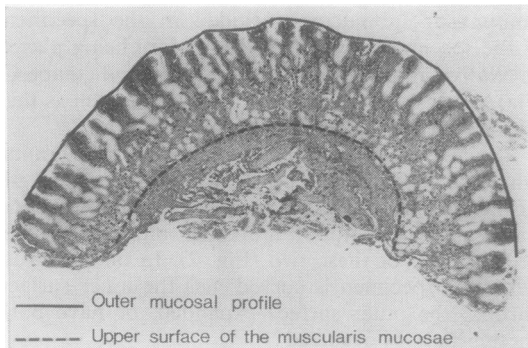


Fig. 2 The lines representing the outer mucosal profile and the upper surface of the muscularis mucosae.

mucosa, especially in the distal stomach, is stretched, and a small, superficial specimen is likely to be obtained (Fig. 3).

The specimens were floated off the biopsy forceps into normal saline and transferred on to filter paper. Some were unfolded and others were allowed to assume their own shape. After fixation in formol sublimate solution they were embedded in wax, and 5μ non-

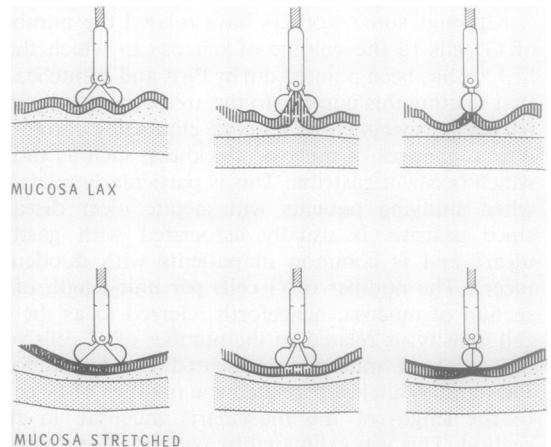


Fig. 3 When the stomach is allowed to relax, the antral mucosa becomes lax, resulting in a biopsy specimen consisting of the full thickness of the mucosa. When the stomach is distended with air, the mucosa becomes taut and the biopsy specimen is likely to be superficial.

serial sections were cut perpendicular to the surface. Three sections from each specimen were mounted on

glass slides, the second and third section being angled at 30° and 60° respectively to the first. (This was to reduce the possible error in length measurement due to angular orientation on the test grid.) Sections were stained for G cells by the immunoperoxidase method described by Piris and Whitehead⁹ and counter stained with light green.

Sections were projected with a Leitz micro-projector at a fixed magnification ($\times 580$) on to a square test grid consisting of two sets of parallel lines crossing each other at right angles. Specimens were considered suitable for quantitative analysis if they consisted of the full thickness of the mucosa and were cut perpendicular to the surface. The total number of G cells, easily recognisable as brown staining cells within the epithelium of the mucosa, were counted in two or three sections from each specimen, depending on its size. The length of the muscularis mucosae and the outer mucosal profile of those sections counted were estimated by counting the number of intersections these made with the lines of the test grid. The G cell density was estimated in three ways:

$$G/I, G/I^1, \text{ and } G/\bar{I}$$

- where G = the number of G cells counted,
 I the number of intersections of the upper surface of the muscularis mucosae with the lines of the test grid,
 I¹ = the number of intersections of the outer mucosal profile with the lines of the test grid,
 and \bar{I} = the mean of I and I¹.

Statistical analysis

Wilcoxon's signed rank sum test was used in experiment 2.

EXPERIMENT 1

Nineteen biopsy specimens which were suitable for quantitative analysis were taken from the prepyloric area of one patient and classified according to their shape (Fig. 1). In six instances, two parts of the same specimen, which were of different shapes as illustrated in Fig. 1, were considered suitable for inclusion in their own right, and the two parts were then analysed separately. The 19 biopsy specimens therefore yielded 25 sets of observations, of which nine were on shape A, nine on shape B, and seven on shape C. G cell densities for shapes A, B, and C were compared, using the three different methods of estimation described above.

Results

Figure 4 shows that there is considerable variation

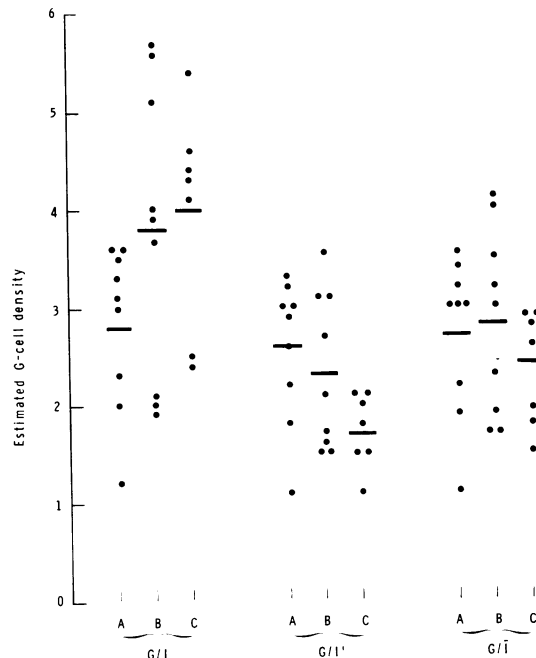


Fig. 4 Experiment 1: Twenty-five biopsy specimens or parts of specimens, from the prepyloric area of one patient, classified according to shape. Shape A is straight, shape B is slightly curved, and shape C is markedly curved. The G cell density has been calculated in three different ways using the length of the muscularis mucosae (G/I), the length of the outer mucosal profile (G/I¹), and the mean of these two lengths (G/ \bar{I}). The last is least affected by variation in the shape of the specimen.

in the G cell density in this patient's prepyloric antrum. However, this is not enough to obscure the fact that the shape of the specimen markedly affects the measured density if this is calculated using the length of the muscularis mucosae (G/I). This is also true, but in the opposite direction, if the length of the outer mucosal profile is used (G/I¹). When the mean of these lengths is used to calculate the G cell density (G/ \bar{I}), the variation is less and appears to be independent of the shape of the specimen.

EXPERIMENT 2

In order to minimise the effects of the intrinsic variability of G cell density in any one patient, 10 biopsy specimens from three different patients were selected for analysis. These were all specimens of complex shape such as that shown in Fig. 1, in which two parts of different shape could be quantified separately, allowing paired analysis of the G cell densities estimated in the three different ways.

Results

Figure 5 illustrates the results of this experiment. Each line represents one biopsy specimen, or pair of shapes, with the estimated G cell density of the straighter part plotted on the left and that of the more curved part on the right. As the degree of curvature of the specimen increased, there was a consistent increase ($P < 0.01$) in the estimated G cell density when this was calculated using the length of the muscularis mucosae (G/I). When the length of the outer mucosal profile was used (G/I'), an increase in the curvature resulted in a decrease in the estimated G cell density in all but one specimen ($P < 0.01$). When the values of G/\bar{I} were compared, however, there was no consistent change with increasing curvature of the specimen ($P > 0.05$), a few specimens giving an increased count, a few

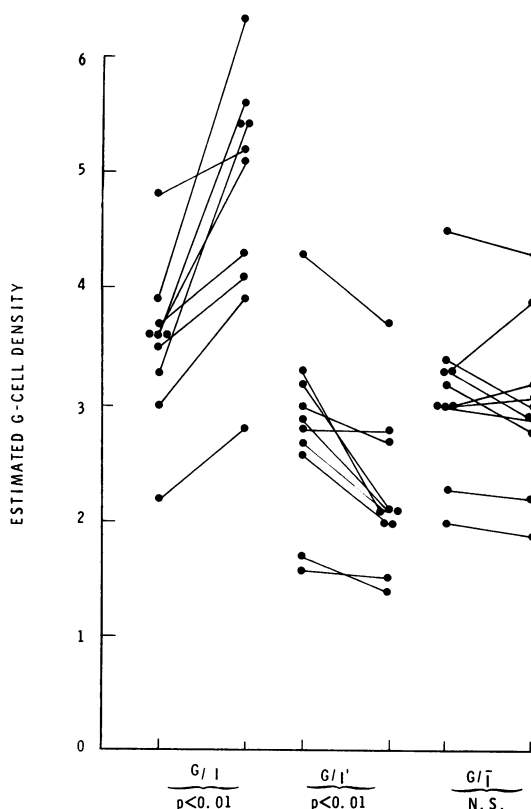


Fig. 5 Experiment 2: Ten biopsy specimens from three patients each consisted of two parts of different shape. The G cell density of each part was calculated in three different ways as before. Each line in the figure represents one specimen or pair of shapes with the estimated density of G cells of the straighter part plotted on the left and that of the more curved part on the right.

a lower count, but the majority giving essentially similar counts.

These results confirm the findings of experiment 1.

Discussion

It is clear from these experiments that when a gastric biopsy specimen, taken through a fiberoptic endoscope, remains curved, the inner surface is contracted and the outer mucosal surface is expanded. The plane of the specimen that is most likely to retain its original length lies between these two surfaces, and its length can be estimated by taking the mean of the lengths of the muscularis mucosae and the outer mucosal profile. By using this derived length (\bar{I}) to estimate G cell density, errors caused by variation in the shape of the biopsy specimen are minimised.

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