

# A method for the quantification of human gastric G cell density in endoscopic biopsy specimens

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**SUMMARY** A previously described method for estimating G cell density in endoscopic biopsy specimens has been modified to take into account observations of the way in which specimens behave when they are deformed and some of the length-measuring characteristics of a grid used to measure the length of tissue profiles. The relationships between particle densities in sections and particle densities in tissue of which the sections are representative are discussed.

A previously described method for quantifying human gastric G cells in endoscopic biopsy specimens relates the number of G cells to the length of the tissue section in which they appear, thus arriving at a G cell density which is independent of variations in mucosal thickness.<sup>1</sup>

This method has been modified to reduce two possible sources of variability inherent in it. The length of the tissue section was originally taken as the length of the line representing the upper surface of the muscularis mucosae. Endoscopic biopsy specimens vary in shape, and it has been shown that when the specimen remains curved, the inner surface is contracted and the outer mucosal or luminal surface is expanded. By relating the number of G cells to the mean of the length of the upper surface of the muscularis mucosae and the length of the outer mucosal profile (the line representing the luminal surface of the specimen), variation in the estimated G cell density due to the shape of the specimen is minimised.<sup>2</sup>

The length of the muscularis mucosae was measured by superimposing a projected image of the section on to a grid consisting of two sets of parallel lines half an inch apart crossing each other at right angles and counting the number of intersections made between the lines of the grid and the tissue profile in question.

The likely number of intersections made between this grid and a curve with directional bias, such as a straight or nearly straight line, is influenced by their angular orientation. When such a curve is repeatedly superimposed at random on the grid and the number of intersections is counted each time, its directional

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bias increases the variability of counts and causes a skew to the right in the resulting frequency distribution histogram. This effect can be virtually eliminated by taking the mean of three measurements, of which the second and third are taken with the curves rotated 30° and 60° respectively from its original position on the grid.<sup>3</sup> By mounting three sections from each specimen on one slide, with the second and third section orientated at 30° and 60° respectively to the first, and determining the G cell density from all three sections, a further potential source of variability is minimised.

## Method

Biopsy specimens obtained from patients undergoing upper gastrointestinal endoscopy with an Olympus GIF-K endoscope, using the standard biopsy forceps (FB-3K), have been used.

The specimens are floated off the biopsy forceps into normal saline and transferred on to filter paper. Each specimen is uncurled with a pair of needles and orientated so that the mucosal surface is uppermost. After fixation in formol sublimate solution, they are embedded in wax, and three 5 µm longitudinal, non-serial sections are cut perpendicular to the mucosal surface. These are mounted on glass slides, the second and third sections being angled at 30° and 60° to the first. They are then stained for G cells by the indirect immunoperoxidase method described by Piris and Whitehead<sup>4</sup> and counterstained with light green.

An image of each section is projected on to the test grid at a fixed magnification. (A Leitz microprojector has been used for this purpose at a magnification of × 580.) Specimens are considered suitable for quantitative analysis if they consist of the full

thickness of the mucosa and are cut perpendicular to the surface.

The total number of G cells ( $G$ ) are counted in all three sections, excluding the extremities of the section if they are not full thickness. The length of the upper surface of the muscularis mucosae and that of the outer mucosal profile of the part of each section containing these G cells are estimated by counting the number of intersections each makes with the lines on the grid ( $I$  and  $I^1$ , respectively). The G cell density is calculated by dividing  $G$  by the mean of  $I$  and  $I^1$ , namely,  $I$ .

A tissue profile is measured by adjusting the stage of the projecting microscope so that the first part of the profile is superimposed on the grid. The number of intersections that this part of the profile makes with the grid is counted, and the field of view is adjusted so that the next part of the profile is superimposed on the grid. This process is repeated until the entire length of the profile is measured in terms of the total number of intersections with the grid.

The orientation of the tissue profiles on the grid depends entirely on their orientation on the slide. As the profiles are measured in all three sections, the effects of angular orientation on the grid are minimised, since the arrangement of the sections on the slide ensures that the orientation of the second and third sections will be about  $30^\circ$  and  $60^\circ$  to that of the first.

**Discussion**

The method described above estimates the number of G cells appearing in a given length of a histological section. Other methods have been described which relate the number of cells to a given area of section.<sup>5 6</sup> The way in which these cell 'densities' derived from tissue sections relate to cell densities in the tissue from which the sections are taken merits discussion.

Stereology is the name recently applied to the study of three-dimensional objects as they are represented in two-dimensional planes that pass through them (true sections). This type of study has been extensively applied to problems in geology and metallurgy, but only relatively recently to the study of the structure of animal tissues.<sup>7</sup>

If the number of particles appearing in a tissue section is related to the area of the section, how does this relate to the number of particles per unit volume of a block of tissue of which the section is representative ( $N_v$ )? Similarly, how does the number of particles per length of section relate to the number per unit area occupied by the block of tissue ( $N_A$ )?

Stereological analysis is difficult, based as it is on the assumption that the tissue section approximates to a true section, in the stereological sense, which has

only two dimensions. The number of particles appearing in a two-dimensional section will depend on their shape and size.<sup>8</sup> For example, no particles will appear in the section if they are infinitely small, however many there are in a block of tissue. In other words, even a small degree of thickness of a three-dimensional tissue section may have a profound effect on the relationship.

The problem is much simpler, however, if the stereological approach is abandoned, and the tissue section is accepted for what it is—a three-dimensional sample of the tissue block. The relationship will still depend on the size and shape of the particles. For simplicity, only spherical particles will be considered.

Imagine a block of tissue of dimensions  $l \times h \times t$  containing  $x$  spherical particles, evenly distributed in the tissue (Fig. 1). The centre of each particle is the

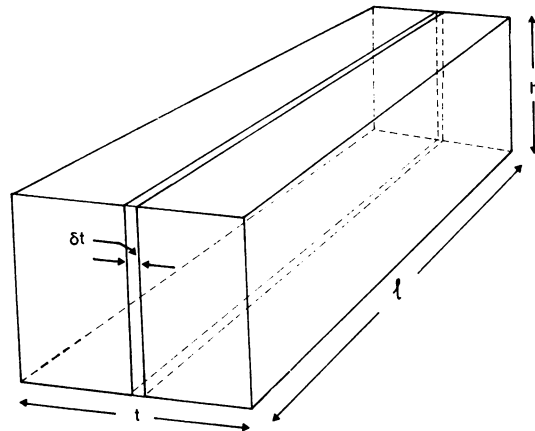


Fig. 1 An imaginary block of tissue of dimensions  $l \times h \times t$ , and a section of that tissue of dimensions  $l \times h \times \delta t$ .

obvious point of location to use. A section of dimensions  $l \times h \times \delta t$  will contain, on average, the centres of

$$x \left( \frac{l \times h \times \delta t}{l \times h \times t} \right) \text{ particles}$$

$$\text{or } \frac{x \delta t}{t} \text{ particles.}$$

However, it will also contain parts of other particles which have their centres in adjacent portions of tissue. How many other particles appear in the section will depend on their size. If the particles are  $d$  in diameter and we assume that any portion of a particle present in the section will be apparent, a further

$$2 \left( \frac{xd}{2} \cdot \frac{1}{t} \right) \text{ particles will appear (Fig. 2)}$$

$$= \frac{xd}{t}$$

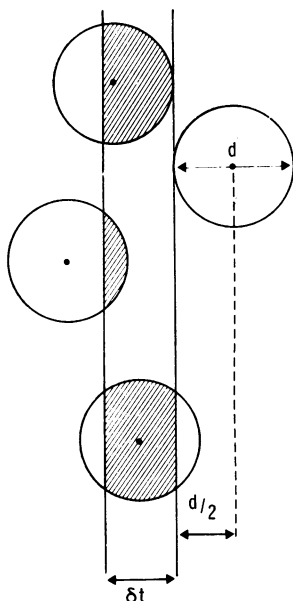


Fig. 2 The number of evenly distributed spherical particles likely to be represented in a section of thickness  $\delta t$  will be not only those whose centres are contained within the section, but also those whose centres lie within half the particle diameter  $d$  on either side of the section.

$\therefore$  the total number appearing in the section

$$= \frac{x\delta t}{t} + \frac{xd}{t}$$

$$= \frac{x}{t} \cdot (\delta t + d)$$

$\therefore$  the number per unit area of section

$$= \frac{\frac{x}{t} (\delta t + d)}{l.h}$$

$$= \frac{x}{l.h.t} \cdot (\delta t + d)$$

$$= N_v(\delta t + d) \dots (1)$$

The number per unit length of section

$$= \frac{\frac{x}{t} (\delta t + d)}{1}$$

$$= \frac{x}{l.t} \cdot (\delta t + d)$$

$$= N_A(\delta t + d) \dots (2)$$

In fact, in practical terms, if only a small portion of a particle is present in the section, it will not be visualised, so that equations (1) and (2) overestimate the relationship. For example, if G cells are assumed to be spheres  $15 \mu\text{m}$  in diameter, the number per unit area appearing in a  $5 \mu\text{m}$  section will be slightly less than

$$N_v(5 + 15)$$

$$= 20 N_v \mu\text{m}^{-2}$$

Similarly, the number per unit length of section will be slightly less than

$$20 N_A \mu\text{m}^{-1}$$

A further reduction in the number of G cells visualised in a tissue section may result from the staining method.

G cells are identified immunologically. This depends upon the anti-gastrin antibody attaching itself to gastrin in the section. If the antibody does not penetrate the tissue, cells such as that labelled 'C' in Fig. 3 will be inaccessible to the antibody and will not stain. The problem would now be reduced to a simple stereological one since a true two-dimensional section only is involved.

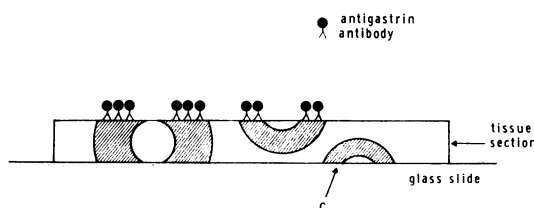


Fig. 3 If the antibody used to identify G cells were unable to penetrate the tissue section, cells such as that labelled 'C' could not be detected.

Only those spheres that are intersected by the plane XY in Fig. 4, representing the upper surface of the histological section, would be stained, and they will have their centres located in tissue  $d/2$  on either side of XY. Thus the number of particles stained would be

$$\frac{xd}{t}, \text{ which is independent of } \delta t.$$

The number per unit area of section would be

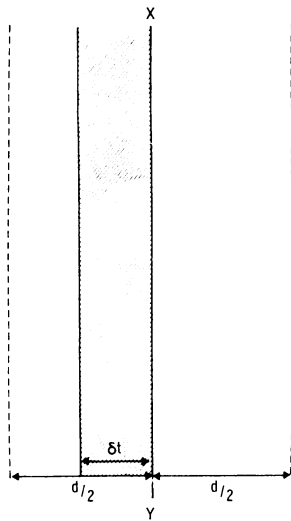


Fig. 4 If only those cells being intersected by the plane representing the upper surface of the section (XY) are detected, their centres will lie within half the particle diameter *d* on either side of the plane XY, irrespective of the thickness of the section.

$$\begin{aligned}
 &= \frac{xd}{t} \cdot \frac{1}{lh} \\
 &= \frac{x}{tlh} \cdot d \\
 &= N_v d. \quad \dots (3)
 \end{aligned}$$

The number per unit length of section would be

$$\begin{aligned}
 &= \frac{xd}{t} \cdot \frac{1}{l} \\
 &= \frac{x}{lt} \cdot d \\
 &= N_A d. \quad \dots (4)
 \end{aligned}$$

The exact relationship between particle densities in sections and particle densities in tissue of which the sections are representative depends on the following:

- 1 the size and shape of the particles;
- 2 the extent to which portions of particles contained in the sections are detected by the staining method; and
- 3 the thickness of the section if this can be penetrated by the 'stain'.

If the particles are spherical and the other factors remain constant, the number seen per unit area of tissue section will be directly proportional to  $N_v$ , and the number per unit length of section will be similarly proportional to  $N_A$ .

The method described above will result in a 'G cell density' which will be approximately directly proportional to the number of G cells per unit area of mucosa of which the tissue sections are representative.

No attempt has been made to quantify the amount of tissue shrinkage that occurs during processing of the biopsy specimens. The method is suited to comparative studies in which absolute values are not relevant, and in this context the amount of tissue shrinkage is of no consequence as long as it is uniform. Since endoscopic biopsy specimens are of similar size and they are fixed, embedded, sectioned, and stained in the same manner, it seems reasonable to assume that the degree of shrinkage is fairly constant.

### Conclusions

The use of endoscopic biopsy specimens to quantify gastric G cell density allows studies to be performed on large numbers of human subjects, each of whom can be studied on more than one occasion. Studies of this kind have been hampered by marked variability in the estimated G cell density. While it is clear that much of this is due to uneven distribution of the G cells, leading to sampling error,<sup>9 10</sup> some variability is bound to be inherent in the methods used.

The method described in this paper results in a 'G cell density' which is independent of variations in mucosal thickness such as those that occur in patients with gastritis. Errors caused by variations in the shape of biopsy specimens are minimised, as are those due to the angular orientation of tissue profiles on the lines of the test grid used to measure their length.

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