Quantitative assessment of the mucosal architecture of jejunal biopsy specimens: a comparison between linear measurement, stereology, and computer aided microscopy*

GR CORAZZA, M FRAZZONI, MF DIXON,† G GASBARRINI

From the III Patologia Medica, Policlinico Universitario S Orsola, Bologna, Italy, and the †Department of Pathology, University of Leeds, Leeds

SUMMARY Fifty jejunal biopsy specimens obtained from normal subjects and from untreated and treated patients with coeliac disease were assessed blindly by three independent observers, each of them using different morphometric techniques—namely, linear measurement, stereology, and computer aided microscopy. In two of 26 control biopsy specimens linear measurement was not possible because of distortion of villi. Highly significant ($p < 0.001$) correlation coefficients were found between the different techniques. With all methods significant differences between controls and patients with coeliac disease and between treated and untreated coeliac patients were found. Only by stereology, however, was there no overlap between results for patients and those for controls. In view of the limitations of linear measurement and the high cost and complexity of computer aided microscopy, we propose that a simple stereological technique using an eyepiece graticule is the method of choice in the quantitative assessment of mucosal architecture in jejunal biopsy specimens.

As jejunal biopsy is a fundamental procedure in the diagnosis of many diseases with malabsorption,¹ the assessment of villus architecture must be as reliable as possible. Greater reliability of morphometry over simple subjective evaluation has recently been shown, and it has been proposed that, whenever possible, qualitative assessment of jejunal biopsy specimens should be coupled with quantitative techniques.²

Up to now either linear^{3,4} or stereological^{5,6} techniques have been employed in jejunal morphometry. These simple methods have recently been criticised and some authors have claimed that they should be replaced by more sophisticated measurements based on computer aided microscopy.⁷⁻⁹ In view of the increasing demand for jejunal morphometry, both histopathologists and gastroenterologists should be aware of the advantages, if any, of a particular method. We have therefore objec-

tively assessed the same series of jejunal biopsy specimens by different morphometric techniques—namely, linear measurement, stereology, and computer aided microscopy.

Material and methods

INTESTINAL BIOPSY SPECIMENS

Fifty jejunal biopsy specimens, selected according to the criteria of Perera *et al*,¹⁰ were studied. All the specimens were taken from the region of the ligament of Treitz using the adult Crosby capsule or the multipurpose tube as part of a routine diagnostic procedure. The biopsies were fixed in buffered formalin, processed by standard methods, and, after paraffin embedding, were cut at 4 μ m thickness and stained with haematoxylin and eosin. At least three sections were taken from the larger biopsies, while multiple sections were cut from the smaller ones. Thus for the entire series there was a mean of 20.9 sections (range 3-56).

The group of 50 patients whose biopsy specimens were included in the study comprised the following:

*This work was presented in part at the XXIV Congress of the Italian Society of Gastroenterology, Bari, Italy, June 1983.

12 patients with untreated coeliac disease, 12 patients with coeliac disease who were on gluten free diets, and 26 subjects whose biopsy specimens were subjectively assessed as normal and who had no evidence of small intestinal disease.

INTESTINAL HISTOLOGY

The jejunal biopsy specimens were assessed blindly by three independent observers (MF, GRC, and MFD) by different morphometric techniques, which measured comparable variables. The fields assessed were chosen at random by each observer.

Linear measurements

Linear measurements (MF) were expressed as villus height to total mucosal thickness ratio (VH/TMT).¹¹ Total mucosal thickness—the distance from the tip of the villus or the flat surface to the muscularis mucosae—and crypt depth—the distance from the muscularis mucosae to the dilatation of the crypt at the base of a villus—were measured for five well orientated villi using an eyepiece micrometer calibrated with a stage micrometer at a magnification of $\times 125$. Villus height was regarded as the difference between total mucosal thickness and crypt depth.

Stereological measurements

Stereological measurements (GRC) were performed according to the method of Dunnill and Whitehead⁶ using a Weibel graticule (Graticules Ltd, Tunbridge Wells, Kent, UK) inserted in the microscope

eyepiece. A record was made of the number of times the lines cut the mucosal surface (c) and the number of hits (h)—that is, end points of the lines falling on the tissue section between the covering epithelium and the muscularis mucosae. At a constant magnification ($\times 125$) the length (l) of each line of the graticule was 1.7×10^{-2} cm. The ratio of c/lh was then calculated and regarded as a measure of surface area to volume ratio (S/V).

Computerised morphometry

The same 50 biopsies were also examined using the Reichert-Jung Kontron IBAS Image Analyser (MFD). The IBAS 1 module was used to measure the ratio of surface length to lamina propria area (SL/LPA). IBAS 1 is a computerised planimeter in which a self illuminated cursor can be superimposed on the microscope field by viewing it through a camera lucida drawing tube attached to a conventional (Leitz Dialux 20 EM) microscope. Each field was viewed through the $\times 16$ objective. To measure surface length the luminal border of the surface epithelium (cutting across the mouths of crypts) was traced from one side of the field to the other using the cursor on a magnetised digitising tablet (Fig. 1). Points along the path of the cursor are digitised by the computer and on completion of the trace the length of the line is given in picture points, so called pixel units. Lamina propria area was measured by drawing around the basement membrane of the surface and crypt epithelium, the sides, and the upper surface of the muscularis mucosae and subtracting

Fig. 1 IBAS 1 measurements. The surface length (SL) is measured by tracing along the luminal border of the surface epithelium. Lamina propria area (LPA) is contained by the solid line drawn around the epithelial basement membrane, the sides of the field, and the superficial aspect of the muscularis mucosae, from which the sum of the areas of the cross sectioned crypts (black) has to be subtracted.

Fig. 2 IBAS 2 measurements. Video image of jejunal biopsy outlined along the surface, the sides, and the superficial aspect of the muscularis mucosae. The total length of this line has been termed perimeter (P).

Fig. 3 IBAS 2 measurements. A second line has been superimposed to identify the lamina propria by drawing along the basement membranes of the surface and crypt epithelium and the superficial aspect of the muscularis mucosae. The area contained by this line is the lamina propria area (LPA).

from the area contained by this line the total area of the cross sectioned crypts (Fig. 1). Lamina propria area was then calculated by the computer and again displayed in pixel units. When these measurements

were completed on one field, two further fields from different parts of the biopsy were selected at random, measured, and the means for each measurement calculated.

We also evaluated the interactive module of the image analyser (IBAS 2), in which an editing facility simplifies the measurement of complex areas such as the lamina propria. The computer analyses a video image generated by a television camera fitted to the microscope, in this case a Grundig FA 70H camera fitted with a plumbikon tube attached to a Leitz Dialux microscope. The operator can interact with the video image by means of specific programs. The components are outlined as before by moving the cursor over the digitising tablet. Once the cursor has been activated a bright green line can be superimposed on the monochrome video image of the mucosa to outline the components to be measured. IBAS 2 will automatically measure a perimeter length and quantify the area contained by the line but will not measure an "unclosed" line such as the mucosal surface length given by IBAS 1. We therefore selected the ratio of mucosal perimeter (Fig. 2) to lamina propria area (P/LPA) for comparison with the other indices. Lamina propria area was measured by tracing around the basement membrane of the epithelium, the sides of the frame, and along the muscularis mucosae and editing out the crypt epithelium (Fig. 3). These measurements were

Table 1 Morphometric analysis of jejunal biopsy specimens from 26 controls

Specimen no	S/V*	VH/TMT†	SL/LPA‡	P/LPA§
1	140.5	0.720	3.696	9.130
2	135.0	0.718	2.174	5.281
3	122.2	0.657	2.018	5.053
4	113.9	0.642	1.634	4.603
5	99.0	0.779	3.349	7.829
6	97.1	0.565	2.078	5.511
7	93.3	0.775	2.077	5.400
8	90.8	0.669	2.128	5.279
9	90.5	0.761	2.422	5.898
10	87.7	0.685	2.856	6.885
11	85.4	0.665	2.219	5.751
12	85.1	0.630	1.253	3.286
13	84.8	0.549	2.148	4.268
14	83.9	0.706	2.726	6.746
15	80.9	—	2.284	5.493
16	77.7	0.556	1.334	3.374
17	76.4	0.715	2.206	5.451
18	74.7	0.673	2.400	6.259
19	74.3	0.737	2.078	5.328
20	70.9	0.636	1.869	5.029
21	70.8	0.611	1.538	4.175
22	69.7	—	2.056	5.154
23	64.5	0.625	1.373	3.678
24	60.0	0.565	2.406	6.036
25	59.2	0.654	1.479	3.162
26	57.6	0.634	1.417	3.842
Mean	87.3	0.663	2.119	5.302

*S/V = surface area to volume ratio (stereological).

†VH/TMT = villus height to total mucosal thickness ratio (linear measurement).

‡SL/LPA = surface length to lamina propria area ratio (computer assisted IBAS 1).

§P/LPA = perimeter to lamina propria area ratio (computer assisted IBAS 2).

Table 2 *Morphometric analysis of jejunal biopsy specimens from 12 patients with untreated coeliac disease*

Specimen no	S/V*	VH/TMT†	SL/LPA‡	P/LPA§
27	17.6	0.348	0.288	1.372
28	15.6	0.155	0.276	1.407
29	15.0	0.226	0.236	1.218
30	13.5	0.262	0.339	1.633
31	10.7	0.043	0.410	1.747
32	10.2	0.262	0.315	1.461
33	9.7	0.172	0.277	1.471
34	9.3	0.090	0.397	1.703
35	6.5	0.275	0.334	1.802
36	5.9	0.175	0.404	1.566
37	4.5	0.045	0.192	1.075
38	4.5	0.042	0.150	1.132
Mean	10.2	0.174	0.301	1.465

*S/V = surface area to volume ratio (stereological).

†VH/TMT = villus height to total mucosal thickness ratio (linear measurement).

‡SL/LPA = surface length to lamina propria area ratio (computer assisted IBAS 1).

§P/LPA = perimeter to lamina propria area ratio (computer assisted IBAS 2).

repeated for two other randomly selected fields and the means were calculated. Each section was viewed using the same $\times 10$ objective, which yielded a final screen magnification of $\times 231$ and meant that the complete frame represented a 0.518 mm² area of the section.

STATISTICS

Two by two comparisons among VH/TMT, S/V, SL/LPA, and P/LPA values were performed using the Spearman's rank correlation test. The significance of the differences between the means for each biopsy group were tested using Wilcoxon's rank sum test.

Results

Tables 1–3 show the results from the 50 patients studied. In two of the 26 (7.6%) control biopsy

specimens the villi were so distorted that the total mucosal thickness could not be measured. Statistical analysis of the results has therefore been performed on only 48 cases. Highly significant correlation coefficients were found when comparing the four techniques (Table 4).

There were significant differences between the means for the control and untreated coeliac disease groups ($p < 0.01$ for all four methods), between the control and treated coeliac disease groups ($p < 0.01$ for all four methods), and between treated and untreated coeliac disease groups ($p < 0.02$ for linear method, $p < 0.01$ for stereological and computer assisted IBAS 1 and IBAS 2 methods).

Discussion

Morphometry is being used increasingly in the assessment of jejunal biopsies both in coeliac

Table 3 *Morphometric analysis of jejunal biopsy specimens from 12 patients with coeliac disease on gluten free diets*

Specimen no	S/V*	VH/TMT†	SL/LPA‡	P/LPA§
39	47.4	0.535	1.279	3.557
40	44.8	0.427	1.061	3.111
41	39.9	0.530	1.204	3.371
42	32.4	0.579	0.890	2.571
43	24.5	0.349	0.520	2.039
44	19.5	0.458	0.669	2.192
45	18.1	0.566	0.451	1.816
46	14.9	0.053	0.283	1.234
47	14.0	0.252	0.324	1.546
48	12.3	0.175	0.333	1.361
49	10.3	0.266	0.385	1.605
50	7.4	0.095	0.428	1.842
Mean	23.7	0.357	0.652	2.187

*S/V = surface area to volume ratio (stereological).

†VH/TMT = villus height to total mucosal thickness ratio (linear measurement).

‡SL/LPA = surface length to lamina propria area ratio (computer assisted IBAS 1).

§P/LPA = perimeter to lamina propria area ratio (computer assisted IBAS 2).

Table 4 Statistical comparison (Spearman's rank correlation test) of the four morphometric techniques

	r_s	P
S/V* v VH/TMT†	0.884	<0.001
S/V* v SL/LPA‡	0.872	<0.001
S/V* v P/LPA§	0.869	<0.001
VH/TMT v SL/LPA	0.892	<0.001
VH/TMT v P/LPA	0.892	<0.001
SL/LPA v P/LPA	0.985	<0.001

*S/V = surface area to volume ratio (stereological).

†VH/TMT = villus height to total mucosal thickness ratio (linear measurement).

‡SL/LPA = surface length to lamina propria area ratio (computer assisted IBAS 1).

§P/LPA = perimeter to lamina propria area ratio (computer assisted IBAS 2).

disease and in many other conditions.

In routine diagnostic histopathology quantitative histology is particularly valuable for evaluating mild mucosal changes and in the serial assessment of biopsy specimens.² Although greater reliability has been claimed for stereological techniques over linear measurements⁶ and for computer aided microscopy over stereological techniques,^{8,9} these methods have not previously been compared.

All the morphometric methods used in this study showed a wide range of values, implying that all four are suitable for quantitative evaluation of varying degrees of mucosal alteration. Moreover, a highly significant correlation between the four methods has been shown and each method effectively distinguished between groups of controls, patients with untreated coeliac disease, and patients with coeliac disease on gluten free diets. Furthermore, there was no overlap in individual results between controls and untreated patients with coeliac disease. As expected, there was considerable overlap between the results for untreated and treated coeliac patients by all four methods and slight overlap between treated coeliac patients and controls by the computer assisted (IBAS 1 and IBAS 2) and linear methods but not by stereology. The absence of overlap between the stereology results for these two groups suggests that this technique is superior to the others in distinguishing abnormal biopsies, but it might be that the statistical assumptions implicit in stereology tend to amplify differences between biopsies.

Despite their overall efficacy, all the morphometric methods we have used are open to criticism. Linear measurements of villus height have been said to be hampered in some instances by defects of histological sectioning^{3,6} and we have confirmed that in a few cases minor inadequacies of the specimen do not permit their use. Furthermore, because the decision as to where the villus ends and the crypt begins is subjective, such measurements

are subject to inevitable interobserver and intraobserver variations. Stereological techniques largely overcome these disadvantages, but the statistical assumptions^{1,2} may be theoretically inappropriate in the estimation of tissue elements or changes that are not randomly distributed.⁸ The good correlations found between the stereological method and the other techniques suggest, however, that the theoretical criticisms levelled at the stereological technique do not limit its practical use and the high interobserver variation claimed recently^{8,9} has been refuted by our previous study.²

Computer aided microscopy allows direct measurement of tissue components on the basis of graphical techniques. Our evaluation of computerized microscopy was extended to the interactive module (IBAS 2) because the ability to produce a visual record of the trace and directly measure complex areas after image editing makes this module theoretically more efficient and less liable to tracing errors. In this mode, however, software restrictions meant that the mucosal perimeter, as opposed to surface length, was measured. Nevertheless, we believe that the mucosal perimeter is a valid measurement in that it is primarily a reflection of villus configuration and is altered to a much lesser extent by changes in overall mucosal thickness and the configuration of the muscularis mucosae. This was borne out by our finding of a highly significant correlation ($r_s = 0.985$) between SL/LPA and P/LPA. On the other hand, because of the high capital cost of image analysis equipment its use in routine and research evaluation of jejunal biopsy specimens would be advisable only if the measurements given by the other two objective techniques were significantly different. With regard to this, we have shown a highly significant correlation between linear and stereological measurements and measurements obtained by computer aided microscopy.

In conclusion, we believe that linear measurement cannot be regarded as the most suitable method for jejunal morphometry because its reliability is questionable and it is more frequently hampered by minor defects of histological sectioning. The cost and complexity of large image analysis machines do not permit their use in routine diagnostic histopathology. It is likely, of course, that the principles of interactive graphics will be further refined, and, ultimately, much cheaper computer systems may be developed. Until such time, however, we propose that the simple and reliable stereological technique used in this study is the method of choice for the routine quantitative assessment of jejunal biopsy specimens.

This work was partly supported by a CNR grant (82 02182 56).

References

- ¹ Brandborg LL. Histologic diagnosis of diseases of malabsorption. *Am J Med* 1979;**67**:990-1006.
- ² Corazza GR, Bonvicini F, Frazzoni M, Gatto M, Gasbarrini G. Observer variation in assessment of jejunal biopsy specimens. A comparison between subjective criteria and morphometric measurement. *Gastroenterology* 1982;**83**:1217-22.
- ³ Shiner M, Doniach I. Histopathologic studies in steatorrhea. *Gastroenterology* 1960;**38**:419-40.
- ⁴ Stewart JS, Pollock DJ, Hoffbrand AV, Mollin DL, Booth CC. A study of proximal and distal intestinal structure and absorptive function in idiopathic steatorrhea. *Q J Med* 1967;**36**:425-44.
- ⁵ Rubin CE, Brandborg LL, Phelps PC, Taylor HC. I. The apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology* 1960;**38**:28-49.
- ⁶ Dunnill MS, Whitehead R. A method for the quantitation of small intestinal biopsy specimens. *J Clin Pathol* 1972;**25**:243-6.
- ⁷ Meinhard EA, Wadbrook DG, Risdon RA. Computer card morphometry of jejunal biopsies in childhood coeliac disease. *J Clin Pathol* 1975;**28**:85-93.
- ⁸ Slavin G, Sowter C, Robertson K, McDermott S, Paton K. Measurement in jejunal biopsies by computer-aided microscopy. *J Clin Pathol* 1980;**33**:254-61.
- ⁹ Tulloh EAM, Baylis JM, Challacombe DN. Automated analysis of morphological change in the duodenal mucosa of children with coeliac disease. *Arch Dis Child* 1981;**56**:860-3.
- ¹⁰ Perera DR, Weinstein WM, Rubin CE. Small intestinal biopsy. *Hum Pathol* 1975;**6**:157-217.
- ¹¹ Scott BB, Losowsky MS. Patchiness and duodenal-jejunal variation of the mucosal abnormality in coeliac disease and dermatitis herpetiformis. *Gut* 1976;**17**:984-92.
- ¹² Chalkley HW, Cornfield J, Park H. A method for estimating volume-surface ratios. *Science* 1949;**110**:295-7.

Requests for reprints to: Dr GR Corazza, III Patologia Medica, Policlinico Universitario S Orsola, Via Massarenti 9, 40138 Bologna, Italy.