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Measurements of intestinal villi in non-specific and ulcer-associated duodenitis—correlation between area of microdissected villus and villus epithelial cell count

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SUMMARY Measurements of villus height, villus area, together with counts of epithelial cells in individual villi, were performed on endoscopic duodenal biopsies from five groups of patients: controls, ulcer-associated duodenitis, mild and severe non-specific (non-ulcerative) duodenitis, cimetidine healed ulcer-associated duodenitis and cimetidine healed non-specific duodenitis. The objectives of the study were two-fold: to establish if epithelial cell count correlated with simpler measurements of villus height or area; and to compare the findings in ulcer-associated and in non-specific duodenitis. Villus area correlated well with epithelial cell count per villus ($r = 0{\cdot}96$); villus height correlated less well ($r = 0{\cdot}66$). When compared with controls, there was a significant decrease in the epithelial cell count per villus in ulcer-associated and severe non-specific duodenitis, but this was confined to the visually inflamed area of the duodenal bulb. After healing of inflammation with cimetidine villus height, area, and epithelial cell count returned to values similar to those in controls. This study confirms that the effects of ulcer-associated and severe non-specific duodenitis on duodenal villi are identical.

Objective measurements of the sizes of small intestinal villi and crypts may be of value in clinical practice—for example, to assess the effects of gluten challenge in a coeliac patient, as well as in research. Most measurements of intestinal villi have been conducted using histological sections, but the technique of microdissection of specimens stained in bulk, previously extensively used in animal work[1] has also been applied to material from human intestine.[2] Villus height may be measured as microns, or as the height of the villus epithelial cell column. However, Wright and his colleagues[3] have criticised the use of villus height as an index of damage to villi, for in a study of jejunal biopsies from coeliac patients they counted the total number of epithelial cells per villus and found that this correlated poorly with the villus length. Unfortunately, it is impractical to use counts of villus epithelial cells in clinical research, for some 30 minutes per villus is necessary to perform this measurement.

Since normal human small intestinal villi are leaf or spade shaped, we reasoned that measurement of the area of a microdissected villus would be likely to correlate well with total villus epithelial cell count. We have therefore compared villus height, villus area, and epithelial cell count per villus in biopsies obtained from the duodenum at endoscopy. We have applied these methods to specimens from patients with duodenitis, both ulcer-associated and non-specific, and controls, in an attempt to detect minor deviations from normal in the duodenal villi of such patients. A previous study has shown that villus height was abnormally low only in visually abnormal, severe duodenitis, ulcer-associated or non-specific.[4]

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Material and methods

The protocol for this study was identical to that described in a previous paper on duodenal mucosal architecture in non-specific and ulcer-associated duodenitis.[4] Briefly, specimens of duodenal mucosa were obtained under direct vision from the first and second parts of the duodenum at upper gastrointestinal endoscopy. Five groups of subjects were studied: controls, ulcer-associated duodenitis, non-specific (non-ulcerative) duodenitis, healed ulcer-associated and non-specific duodenitis on treatment with cimetidine. Specimens were taken from five

subjects in each group. Controls were subjects in whom the only endoscopic abnormality was hiatus hernia. Non-specific duodenitis patients were further subdivided into mild and severe categories on the basis of endoscopic appearances. Those with mild hyperaemia and swelling of the mucosa with slight contact bleeding were classified as mild duodenitis. Intense hyperaemia and mucosal swelling, when associated with spontaneous bleeding and multiple erosions were classified as severe duodenitis. Cimetidine treated cases were selected to include those in whom visual healing of inflammation had taken place after six to twelve weeks of treatment with conventional dosage of cimetidine.

In the control, healed ulcer-associated and healed non-specific duodenitis subjects biopsies were taken from any part of the bulb and distal second part of the duodenum. In the ulcer-associated duodenitis, mild and severe non-specific duodenitis subjects two biopsies were taken from the duodenal bulb, one from visually inflamed and the other from visually normal areas; in addition biopsies were taken from the second part of the duodenum.

Specimens were fixed immediately after collection by immersion in Clarke's fixative (75% ethyl alcohol, 25% glacial acetic acid). After four hours at room temperature the specimen was transferred to 75% ethyl alcohol in water and stored.

Specimens were stained in bulk by the Feulgen reaction. These were kept in 50% ethyl alcohol for 10 min; in tap water for 10 min with three changes; in molar hydrochloric acid for 6 min at 60°C in a water-bath; in tap water for 10 min with three changes; in Schiff's reagent at room temperature for 45 min; in tap water for 10 min with three changes; in Giemsa's stain (diluted 1/10 in water) at room temperature for 30 min. The specimen was then rinsed in water to remove excess stain.

MICRODISSECTION AND MEASUREMENTS

The specimen was placed in tap water under a dissecting microscope. With fine forceps and a cataract knife, a number of strips (length $\approx$ 2 mm) and one or two villi thick were cut free. Individual villi were separated from these strips with a fine needle, care being taken to cut the villus at its base. A single villus was then put in a drop of 45% acetic acid on a slide and covered with a cover-slip (Fig. 1). Its length was then measured with an eye-piece graticule under a light microscope. Its image was then projected on a screen from a fixed distance and the outline of the villus mapped out on paper. Afterwards, the area of the villus was measured by using a planimeter.

The same villus was then pressed gently under the cover-slip with the tip of a needle to spread the cells

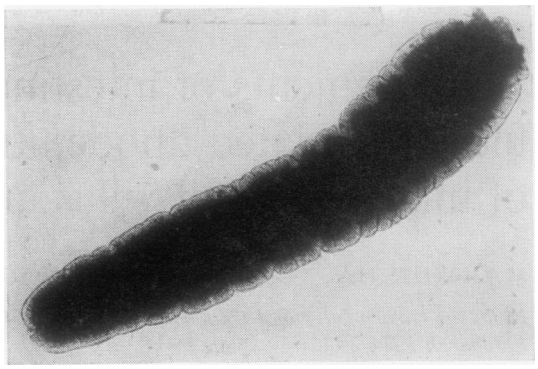

Fig. 1 *Microdissected single villus. Schiff and Giemsa* × *160.*

out uniformly (Fig. 2). The nuclei of the epithelial cells (oval shaped, paler stained) could be readily distinguished from the nuclei of plasma cells, lymphocytes, fibroblasts, polymorphonuclear cells (Fig. 3). The number of epithelial cells was then counted field by field until all the fields on the slide had been examined. Total cell counts were performed on 10 villi from each specimen.

Statistical methods

Kruskal and Wallis' test and Wilcoxon's sum of ranks test were applied for comparison of values for the various measurements.

Results

Preparations of duodenal biopsies from the different sites were adequate in every case, although it was

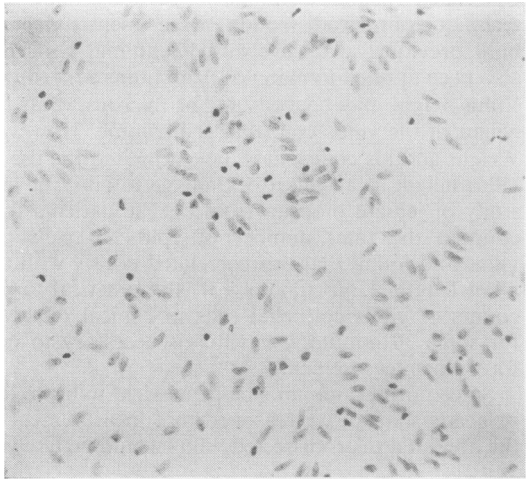

Fig. 2 *Squashed villus cells. Schiff and Giemsa* × *125.*

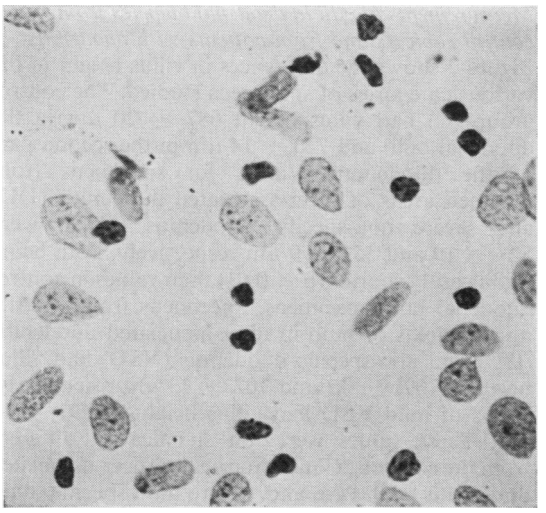

Fig. 3 *Squashed villus cells. Schiff and Giemsa × 500.*

often necessary to examine two biopsies from a site in order to obtain 10 villi suitable for measurement and epithelial cell count. The villi showed considerable variation in shapes and sizes in biopsies from both normal and abnormal areas. It was not possible to differentiate normal and inflamed areas on the basis of appearances of the villi.

Correlation between villus cell count, villus height and villus area

The relations between measurements of villus size by cell count, projected villus area and villus height are here illustrated by analysis of results for three biopsies of duodenum from control subjects. For purposes of simplicity, the term villus area will be used to denote the projected villus area in the results and discussion sections.

Total number of epithelial cells per villus was 2138 ± 90 in the duodenal bulb and 2118 ± 79 in the second part of the duodenum (these values and those given in the rest of the results section are all expressed as mean and standard error of the mean of the group concerned). Coefficient of correlation (r) was 0·66 between height of the villus and the number of epithelial cells per villus in control specimens from the duodenal bulb ($p < 0·001$). The value of (r) between the area of villi and the number of epithelial cells per villus was 0·96 in the control duodenal bulb specimens ($p < 0·001$). Calculation of coefficient of correlation in the other groups of specimens between villus height and number of epithelial cells per villus and villus area and number of epithelial cells per villus gave results which were similar to those obtained in the control duodenal bulb specimens. Thus in each group the epithelial cell population in a villus correlated well with villus area but the correlation with villus height was less satisfactory. Figures 4, 5, and 6 shows the correlation between the three parameters, villus height, villus area and

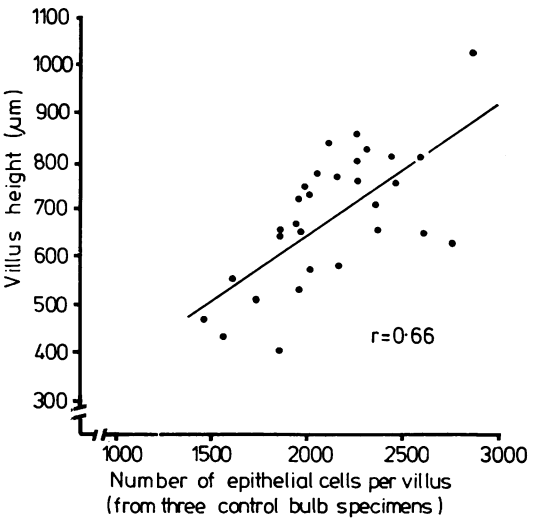

Fig. 4 *Correlation between villus height and number of epithelial cells per villus.*

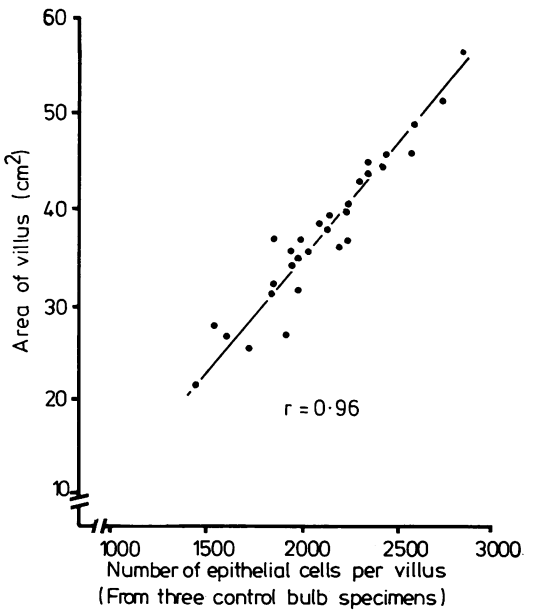

Fig. 5 *Correlation between villus area and number of epithelial cells per villus.*

number of epithelial cells per villus in three control duodenal bulb specimens.

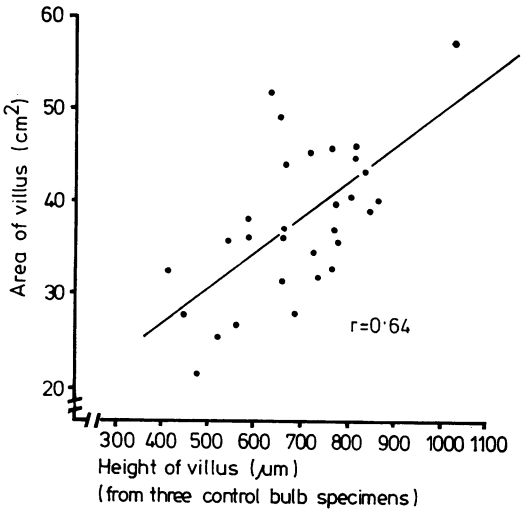

Fig. 6 *Correlation between villus height and villus area.*

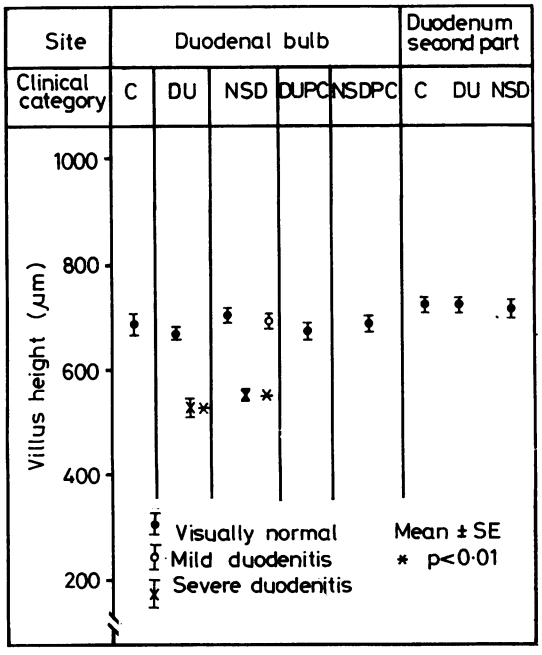

Fig. 7 *Measurement of villus height in μm. C = controls, DU = ulcer-associated duodenitis, NSD = non-specific duodenitis, DUPC = healed ulcer-associated duodenitis after cimetidine treatment, NSDPC = healed non-specific duodenitis after cimetidine treatment.*

Measurements of villi in duodenal biopsies from control subjects and from patients with duodenitis

Figure 7 shows the differences in villus height in the various categories of specimens studied. The control group (C) had villus height 687 ± 20 μm in the duodenal bulb and 720 ± 14 μm in the second part of the duodenum. Values for specimens from inflamed areas of ulcer-associated duodenitis (DU) and severe non-specific duodenitis (NSD) were 527 ± 19 and 552 ± 9 μm respectively, both being significantly shorter ($p < 0.01$) than values in control duodenal bulb specimens. Specimens from visually normal areas of bulb in ulcer-associated duodenitis (DU) and non-specific duodenitis (NSD) had villus height of 691 ± 12 and 702 ± 13 μm respectively. Areas of mild NSD had villus height of 691 ± 14 μm. These values were not significantly different from the controls. Cimetidine healed ulcer-associated duodenitis (DUPC) and severe non-specific duodenitis (NSDPC) had villus height not significantly different from the controls, the values being 671 ± 13 and 684 ± 14 μm respectively. In the second part of the duodenum, villus height in biopsies from DU and NSD cases were similar to the control values, the figures being 722 ± 13, 715 ± 14, and 720 ± 14 μm respectively.

Similar differences emerged in comparison of villus area between the different groups (Fig. 8). The control value for duodenal bulb 33 ± 2·1 (in cm^2) was significantly greater than values for inflamed areas of DU (17·3 ± 1·6 cm^2) and severe NSD (18·8 ± 0·9 cm^2), the value for p being less than 0·01 in both cases. Values for uninvolved bulb in DU and NSD, mild NSD, DUPC and NSDPC were 31·4 ± 1·7, 32·4 ± 1·9, 32·3 ± 2, 29·5 ± 1·9, and

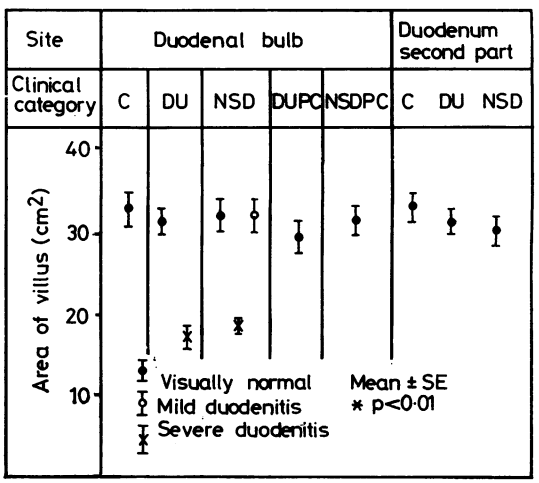

Fig. 8 *Measurements of villus area. Abbreviations as in Fig. 7.*

31·6 ± 1·8 (in cm^2) respectively. None of these values was significantly different from the control value. In the second part of duodenum, values for control, DU and NSD were 33·3 ± 1·9, 31·4 ± 1·6, and 30·4 ± 1·9 (in cm^2) respectively, showing no significant difference from each other.

The number of epithelial cells per villus was 1318 ± 77 in the specimens from inflamed areas of DU and 1373 ± 40 in inflamed areas of NSD (Fig. 9). These were significantly differerent ($p < 0·01$)

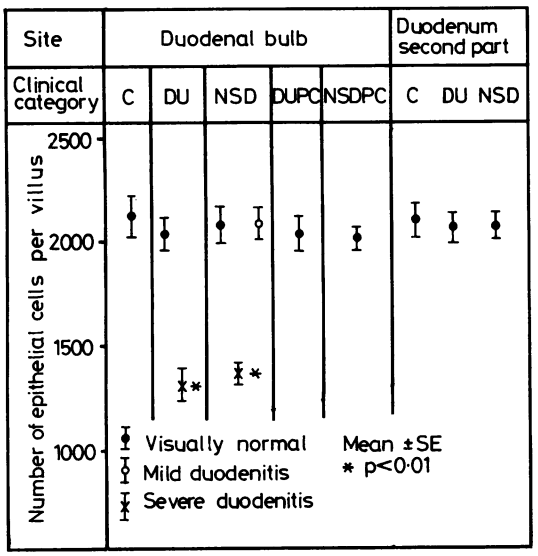

Fig. 9 *Villus epithelial cell counts. Abbreviations as in Fig. 7.*

from control values of duodenal bulb (2138 ± 90). The number of epithelial cells per villus in visually normal areas of DU, NSD and inflamed areas of mild NSD were 2043 ± 74, 2082 ± 82, and 2106 ± 81 respectively. None of these was significantly different from the control value. No significant difference from controls was found in the numbers of epithelial cells per villus in DUPC and NSDPC, the values being 2045 ± 81 and 2024 ± 67 respectively. In the second part of the duodenum, the number of epithelial cells per villus was 2078 ± 68 for DU, 2082 ± 82 for NSD. These values were similar to the control value from second part of duodenum, this being 2118 ± 79.

Discussion

Microdissection of endoscopically obtained biopsies of the duodenal mucosa provides a simple and inexpensive method for performing measurements of intestinal mucosal villi and crypts. We have now shown that although biopsies are small it is possible to separate individual villi and count the number of epithelial cells per villus. We have confirmed the previous report[3] that in disease, the reduction in the number of epithelial cells per villus is of greater magnitude than the corresponding reduction in villus height. In circumstances where there is considerable variation in shape and size of villi, as in the normal duodenum, direct enumeration of the epithelial cell population of a single villus is one way to obtain precise information on the villus size. However, since measurement of the villus area correlates very well with the total number of epithelial cells in the villus, villus area is likely to be a more practicable measurement for clinical investigation and research. We have also confirmed that the villus height correlates less well, although significantly, with villus cell count.

Duodenitis may be defined as a clinical entity on the basis of radiological appearances of duodenum, by visual examination at endoscopy, and by changes at histological examination of duodenal biopsies. On the basis of subjective assessment at endoscopy and histopathology, duodenitis has often been classified as mild or severe. For the purposes of this study, we have chosen to define duodenitis on the basis of visual appearances at endoscopy (see methods). That this definition is valid has been demonstrated by objective measurements of villi and crypts, counting of mitotic figures in crypts[4] and of intraepithelial and lamina propria inflammatory cellular infiltrate. These studies have shown that increased cellular infiltrate and mucosal architecture abnormalities are found in biopsies from visually inflamed areas of duodenitis and are normal in visually normal areas. Now by using direct enumeration of epithelial cells per villus we have confirmed our previous observation, based on measurement of villus height, that there is reduction in villus size in non-specific duodenitis as well as in ulcer-associated duodenitis and that the changes are identical in the two diseases.[4]

The reduction in villus cell count was confined to visually abnormal areas of the duodenal bulb and was not present in the second part of the duodenum. Furthermore, villus cell population returned to normal after healing of inflammation in non-specific or ulcer-associated duodenitis with cimetidine.

We are grateful to Professor Nicholas Wright for his advice on the method of epithelial cell counting and to Miss Frances Allan for her help with the setting up of the technique. We acknowledge the help of the endoscopy theatre staff for their co-operation with the collection of the biopsies and of the Association

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References

[1] Clarke RM. Control of intestinal epithelial replacement: lack of evidence for tissue-specific blood-borne factor. *Cell Tissue Kinet* 1974;7:241-50.
[2] Ferguson A, Sutherland A, Macdonald TT, Allan F. Technique for microdissection and measurement in biopsies of human small intestine. *J Clin Pathol* 1977;**30**: 1068-73.
[3] Wright N, Zucoloto S, Bramble M, Record C. Assessment of villus and crypt population sizes in human small intestinal biopsies. *Gut* 1979;**20**:A 921.
[4] Hasan M, Sircus W, Ferguson A. Duodenal mucosal architecture in non-specific and ulcer-associated duodenitis. *Gut* (in press).

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