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Morphometric analysis of small intestinal mucosa IV. Determining cell volumes

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Abstract. This study was concerned with the measurement of volumes of surface epithelial cells in human small intestine, both in disease-control subjects and, for comparison, in patients with gluten sensitivity. Four procedures were employed, of which two were geometrical, based on cylindrical or truncated conoid models. The third method evolved from the proportionality of area to volume, and required determination of cellular and nuclear profile areas, and an estimation of nuclear volume based on models conforming to (1) a prolate spheroid, or (2) a cylinder with hemispherical caps. This procedure appeared to underestimate enterocyte volumes and failed to reveal volume differences between controls and gluten-sensitive individuals. Finally, a fourth method was devised, based on traditional intraepithelial profile counts per hundred enterocyte nuclei, calculation of surface epithelial volume and of the absolute number of lymphocytes contained therein. Enterocyte volumes appeared to be overestimated twofold by this procedure compared with the first two geometric methods. The results of this study indicate that the cuboidal-type enterocyte profiles typical of the untreated mucosa in gluten sensitivity are a reflection of cells with a reduced volume. From the number of enterocytes and the absolute lymphocyte population present within a morphometrically defined volume of surface epithelium, the ratios of intraepithelial lymphocytes to enterocytes were found to be 50% less than conventional density-profile counts.

Key words: Enterocyte – Morphometry – Cell volume – Intraepithelial lymphocyte – Coeliac

Introduction

One of the striking features of the mucosal lesion in gluten sensitivity is the marked reduction in height profile of surface enterocytes, whose appearances change from tall columnar to lower cuboidal cells (Rubin et al. 1960; Shiner and Doniach 1960). This phenomenon has traditionally always been taken to imply (1) a direct noxious effect of gluten on surface enterocytes (Frazer 1960) and (2) the presence of small cells (microenterocytes), analogous to the microcytic erythrocyte of severe iron deficiency anaemia (Booth 1968).

The factors which determine the size and shape of polarised epithelial cells are numerous, although they may be classified broadly into (1) morphogenetic [Kedinger et al. 1988; McDonald 1989; Molitoris and Nelson 1989; Rodriguez-Boulan and Nelson 1989 (cell-cell and cell-matrix interactions)], (2) physiological (Sachs 1989; MacLeod et al. 1991) and (3) pathological (Table 1). In various enteropathies, cell shape may be largely dependent on matrix connections and the availability of nutrients and blood, the supply of which may be endangered by the underlying pathology of the mucosal lesion and malabsorption process. Although for many years the presence of cuboidal enterocytes in the advanced gluten-sensitive lesion has, if somewhat uncritically, been linked directly to gluten-induced damage, this view is strictly untenable since identical changes affect surface enterocytes in the presence of avillous mucosal flattening in tropical diarrhoea-malabsorption syndrome, giardiasis, immunodeficiency and immunoproliferative small intestinal disease (Marsh 1987). Thus there may be something intrinsic to the process, and mechanism(s) of mucosal flattening, rather than in aetiology of each specific enteropathy, which causes a reduction in the height profiles of surface enterocytes.

Despite the fairly large literature on this subject, it is surprising that all previous cell measurements have been confined to the two-dimensional realm of the tissue section. Yet it is evident, for example, from studies of experimental wound-healing (Florey and Harding 1935; McMinn and Mitchell 1954), that alterations in cell shape and volume do occur. These may occur with great rapidity, and hence influence the appearance of sectioned profiles of cells caught up in these processes.

We therefore decided to use computerised image-analysis to measure enterocyte volumes and to determine

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Table 1. Some factors affecting epithelial cell shape

1 Morphogenetic	Cell:cell	Kedinger et al. 1988; McDonald 1989; Molitoris and Nelson 1989; Rodriguez-Boulan and Nelson 1989
2 Physiological	Ion-channel fluxes mechanosensors	Sachs 1989; MacLeod et al. 1991
3 Pathogenetic		
(i) Anti-mitotic agents:	Aminopterine, 5-fluorouracil	Zamcheck 1960; Williams 1963
(ii) Noxious agents:	Lactic acid, ZE syndrome, alcohol, laxatives and bowel cleansing agents	Townley et al. 1964; Hamilton 1967; Shimoda et al. 1968; Hermos et al. 1972; Gaginella and Phillips 1976; Meisel et al. 1977; Saunders et al. 1977; Pockros and Foroozan 1985
(iii) Transplantation:	Bowel exteriorisation, transposition, grafting	Stephens et al. 1964; Gracey et al. 1971; Deschner et al. 1973; Elson et al. 1977; Pabst and Kamran 1983
(iv) "Hydrostatic" forces:	Oedema, raised venous pressure, increased secretion	Elliott et al. 1970; Saunders et al. 1975; Granger et al. 1976; Buele et al. 1983
(v) Enteropathies:	Graft-versus-host, gluten sensitivity, tropical sprue, giardiasis, etc.	Chacko et al. 1961; Yardley et al. 1962, 1964; Holmes et al. 1971; Khojasteh 1987
(vi) True mucosal atrophy:	Chronic radiation damage, Chronic ischaemia	Whitehead 1972; Fisher and Myers 1984; Morgenstern 1984; Fajardo 1989
(vii) Pharmaceutical agents:	Colchicine, mefenamic acid	Clark and Harland 1963; Batt 1989

whether the reduced enterocyte profiles seen in untreated gluten-sensitive mucosae were truly representative of smaller cells.

Materials and methods

The subjects consisted of ten disease-control patients (7 male/4 female; age range 23–48 years) in whom a jejunal biopsy was performed as part of their diagnostic work-up, but in whom gluten-sensitivity was excluded by the presence of a normal biopsy. There were also 11 healthy volunteers (5 male and 6 female; all under 30 years of age) recruited from the medical and paramedical staff of this hospital. These 21 control mucosae were compared with jejunal mucosae obtained from 20 patients (8 females, aged 22–58 years and 12 males, aged 25–72 years) with untreated gluten sensitivity. They were drawn from the Coeliac Clinic at Hope Hospital, and all were shown to be gluten-sensitive by their subsequent improvement on a gluten-free diet.

Mucosal biopsies, obtained via a Watson capsule located fluoroscopically to the first loop of jejunum, were rapidly retrieved and flooded with 2.5% ultrapure glutaraldehyde in 0.1 M cacodylate buffer for 2–3 min. After initial hardening, specimens were transferred to fresh fixative for a further 60 min and then post-osmicated (in 1% osmium tetroxide) dehydrated through an ascending ethanol series and embedded in Araldite epoxy-resin. Sections 1 μm thick were cut on Reichert OMU-3 ultramicrotome with glass knives, stained with buffered toluidine blue and mounted on DPX slides.

Two geometric methods were used to determine cell volume based on an assumed model of enterocytes as (1) cylinders or (2) truncated inverted conoids. Cylinder representation is the simplest

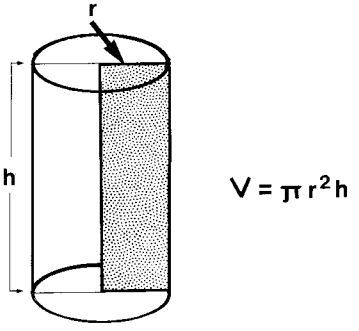

Fig. 1. This model (model 1) depicts an enterocyte as a cylinder, whose volume is dependent on measurement of profile height, *h* and one half, *r*, of mean width measured at brush border and at contact with basal lamina

model of cell volume which is described by the rotation of a planar rectangle (Fig. 1) through 360°. The sides of the cylinder are parallel and both ends assumed (for convenience) to be circular. The length of the cylinder is the vertical profile (sectioned) height of the enterocyte (*h*) and its volume is $\pi r^2 \cdot h$ where *r* is one-half the mean profile cell width: the latter is derived from the widths of the cell at the brush border (w_1) and at its contact with the basal lamina (w_2). Thus

$$r = \frac{w_1 + w_2}{4}$$

In the inverted truncated conoid model the space occupied is described by the rotation of a truncated right-angled triangle through 360° (Fig. 2). The volume, *V*, thereby described is given by the equation:

$$V = \pi \frac{h}{3} [r_1^2 + (r_1 \cdot r_2) + r_2^2]$$

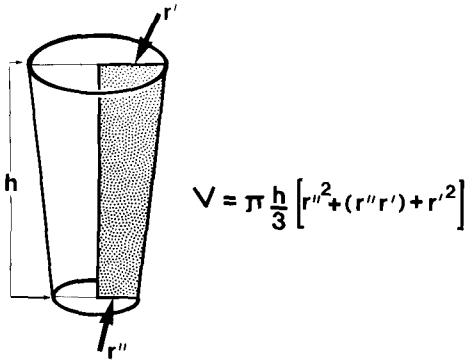

Fig. 2. In this conceptualised model of an enterocyte (model 2), its shape is considered to be an inverted, truncated conoid. Its volume is derived from the profile height, h, and one-half values (r^{I}, r^{II}) for the upper (brush border) and lower (basal lamina) cell widths, respectively

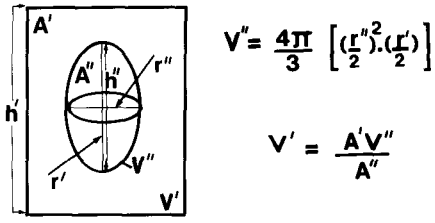

Fig. 3. This approach to cell volume determination (method 3) is based on proportionality of area: volume ratios. The cell (A^{I}) and nuclear (A^{II}) area profiles are obtained directly from the tissue section. Nuclear volume is derived by two methods (see Fig. 4). Here nuclei are assumed to be prolate spheroids, the volume of which is dependent on long (major) axis (i.e. nuclear height) (r^{I}) and on one-half shorter (minor) axis, i.e. $r^{II}/2$. Given these three values, the unknown part of the proportionality ratio (volume of enterocyte, V^{I}) can be calculated

The area-volume ratio method uses a previously described morphometric procedure (Hecker et al. 1972) based on the mathematical proportionality between cell area and volume (Weibel 1979). For any sectioned cell profile, the ratio of the nuclear to cytoplasmic areas (A_{NU}:A_{ENT}) is directly proportional to the nuclear to cytoplasmic volumes (V_{NU}:V_{ENT}; Fig. 3). From a two-dimensional tissue section, sufficient information can be extracted to measure the cytoplasmic to nuclear ratio and nuclear volume. In order to calculate nuclear volume it is necessary to measure the long (x) and short (y) axes, if nuclear shape is assumed to approximate that of a prolate ellipsoid. Thus:

$$V_{NU}=\frac{4}{3}\pi\left[x\cdot\left(\frac{y}{2}\right)^2\right] \quad \text{and} \quad V_{ENT}=V_{NU}\left[\frac{A_{ENT}}{A_{NU}}\right]$$

The method for determining the volume of a prolate ellipsoid was confirmed with the use of intact egg-shells and the volumes of water required to fill them. However, all nuclei do not have ovoid profiles, but instead appear cylindrical with hemispherical caps. As an alternative method, volumes were determined by adding the volume of a cylinder [height (h) × area (πr^2)] where r is one-half of the width of the nucleus (w) and the volume of its two hemispherical caps is

$$(\tfrac{4}{3}\pi r^3) \quad \text{or} \quad 4.2r^3$$

as shown in Fig. 4.

The volume of an enterocyte was based on intraepithelial lymphocyte (IEL) density counts per 100 enterocytes (Ferguson and Murray 1971), in which 1000 enterocyte nuclei were, in practice, enumerated (Niazi et al. 1984). From the absolute number of IEL determined by computer ($N_{V,SE}$), the total number of enterocytes was calulated from the IEL density count, since n IEL are related to 100 enterocytes: the number of enterocytes (N_{ENT}) in a given surface epithelial volume (V_{SE}) is $\frac{N_{V,SE}\cdot 100}{n}$ and hence the average volume of an enterocyte (V_{ENT}) is $\frac{V_{SE}}{N_{ENT}}$ μm³.

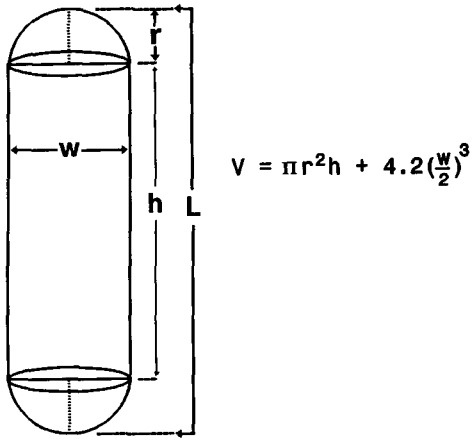

Fig. 4. In addition to assuming nuclei are prolate spheroids, they may also be conceived as cylinders capped by hemispherical poles: combined, these latter form a sphere whose volume radius (r) is one-half mean nuclear width (w). The volume of the intervening cylinder is again dependent on r, and its height (h), which is overall length of nucleus, L, minus $2r$ (w)

This calculation is based on data for (1) surface epithelial volumes (V_{SE}) per 10^4 μm² of muscularis mucosae, (2) total „absolute" numbers of IEL ($N_{V,SE}$) occupying V_{SE} per specimen and (3) the density ratio of IEL profiles per 1000 enterocyte nuclear profiles, expressed as a percentage. We were also able to compare IEL density counts (%) with our own morphometric data, based on the percent ratio of $N_{V,SE}$ to N_{ENT} within V_{SE}, as determined per specimen.

Results

Since many of these values were previously unknown, the data were first analysed for normality of distribution. For most, the degree of kurtosis was tolerable and parametric tests were employed, the main exceptions being volume data both for enterocytes and enterocyte nuclei which were therefore transformed logarithmically. Since IEL density counts are non-normally distributed, all values and calculations pertaining to such data (Table 2) were also log-transformed.

There was a close relationship between the values obtained by method 1 (cylinder representation) and method 2 (inverted truncated conoid), the log-transformed means and 95% confidence limits being, for controls: 770 (475–1247) μm³ and 800 (510–1260) μm³; and for untreated gluten-sensitive mucosae: 590 (385–910) μm³ and 610 (400–930) μm³, respectively ($P=0.001$) indicating that the surface enterocytes in a flat mucosa are truly reduced in size (Table 2). Values determined by method 3 (area: volume ratio) which were dependent on

Table 2. Summary of morphometric data for enterocytes

	Disease-controls ($n=21$)	Untreated gluten-sensitivity ($n=20$)	P value
Cell			
Volumes (μm^3)[a]			
Method 1	770 (475–1247)	590 (385–910)	0.001
Method 2	800 (510–1260)	610 (400–930)	0.001
Method 3 (i):	527 (274–1014)	520 (225–1190)	NS
(ii):	494 (313–776)	437 (270–710)	NS
Method 4:	1547 (850–2810)	1150 (650–2040)	<0.005
Area (μm^2):	182 (130–240)	152 (113–190)	<0.001
Width (μm):	5.13 (4.10–6.20)	4.68 (3.75–5.83)	0.01
Height (μm):	37 (30–43)	33 (27–39)	0.01
Ratio height/width:	7.3 (5.2–9.3)	7.2 (5.0–9.3)	NS
Nuclei			
Volumes (μm^3)[a]			
As prolate spheroid:	94 (45–198)	118 (48–293)	NS
As cylinder:	91 (58–143)	100 (59–168)	NS
Area (μm^2):	33 (24–45)	34 (25–46)	NS
Width (μm):	3.3 (2.6–4.0)	3.5 (2.6–4.5)	NS
Height (μm):	11.6 (9.6–14.0)	11.5 (9.8–13.5)	NS

[a] Denotes logarithmic transformation
NS, not significant
Figures in parentheses are 95% confidence limits

calculations of nuclear volumes either as prolate spheroids, or as cylinders with hemispherical caps, were comparatively smaller, being 527 (274–1014) μm^3 and 494 (313–776) μm^3 for controls, and 520 (225–1190) μm^3 and 440 (270–710) μm^3 in untreated gluten sensitivity; neither set of values differed significantly. This disparity may have arisen because there were no major differences in nuclear volumes between disease-controls, (1) 94 (45–198) μm^3 and (2) 91 (58–143) μm^3 and gluten-sensitive individuals, (1) 118 (48–293) μm^3 and (2) 100 (59–168) μm^3.

Corresponding values for nuclear height, width and area were: 11.6 (9.6–14.0) μm and 11.5 (9.8–13.5) μm; 3.3 (2.6–4.0) μm and 3.4 (2.6–4.5) μm; 33 (24–45) μm^2 and 34 (25–46) μm^2, respectively: none of these values was significantly different (Table 2).

The relationship between profile heights and widths was compared with their respective volume determinations. Data for enterocyte heights were: for controls 37 (30–43) μm and for gluten sensitivity 33 (27–39) μm ($P=0.01$); and for enterocyte widths, controls 5.13 (4.10–6.20) μm and gluten sensitivity 4.68 (3.75–5.83) μm ($P=0.01$). Surprisingly, cell heights showed less significant correlation on cell volume ($r=0.4849$: $r^2=0.2351$, $P<0.05$ by method 1 and $r=0.5214$: $r^2=0.2718$, $P<0.001$ by method 2) whereas cell widths were very highly significantly correlated with cell volume ($r=0.9222$: $r^2=0.8506$, $P<0.0001$ by method 1 and $r=0.9124$: $r^2=0.8324$, $P<0.0001$ by method 2), all correlations being based on combined values for controls and gluten-sensitivity (Fig. 5). The mean ratios between profile height and width for controls and gluten-sensitive subjects were identical (7.3 and 7.2 respectively) suggesting that similar changes in geometry take place as the cell volume is progressively reduced. Thus the reasons for the disparity between cell volume, and their related dimensions of height and width, are not immediately apparent.

Values for V_{SE} (per 10^4 μm^2 muscularis mucosae) for control and gluten-sensitive individuals [2.3 (1.5–3.6) $\times 10^6$ μm^3 and 0.4 (0.2–0.6) $\times 10^6$ μm^3, respectively] were consistent with previously established data from our laboratory. The absolute populations of IEL ($N_{V,SE}$) contained therein [derived from observed profiles overlying the test area; mean lymphocyte nuclear diameters (D_N) of 4.9 (4.2–5.6) μm and 5.2 (4.6–5.9) μm respectively, and an estimated section thickness of D_N+t (where $t=1$ μm for thickness of epon section) were 350 (275–450) μm for controls, and 190 (150–240) μm for untreated gluten-sensitive subjects, thus again confirming previous results (Guix et al. 1979; Marsh 1980; Niazi et al. 1984; Jenkins et al. 1986)].

From $N_{V,SE}$ and IEL count densities, volume of enterocytes (method 4) was 1547 (850–2810) μm^3 for disease-control mucosae, and 1150 (650–2040) μm^3 for gluten-sensitivite mucosae. For both groups of subjects, these values (Table 2) were significantly different ($P<0.005$), but were also increased approximately two-fold over the values obtained by geometric methods (1 and 2).

From the calculated data, three additional derived values were obtained. The first was the average number of enterocytes in villous (surface) epithelium (V_{SE}/V_{ENT}). For control mucosae this is approximately 3000 [3040 (1960–4720) and 2930 (1935–4435)] and for untreated

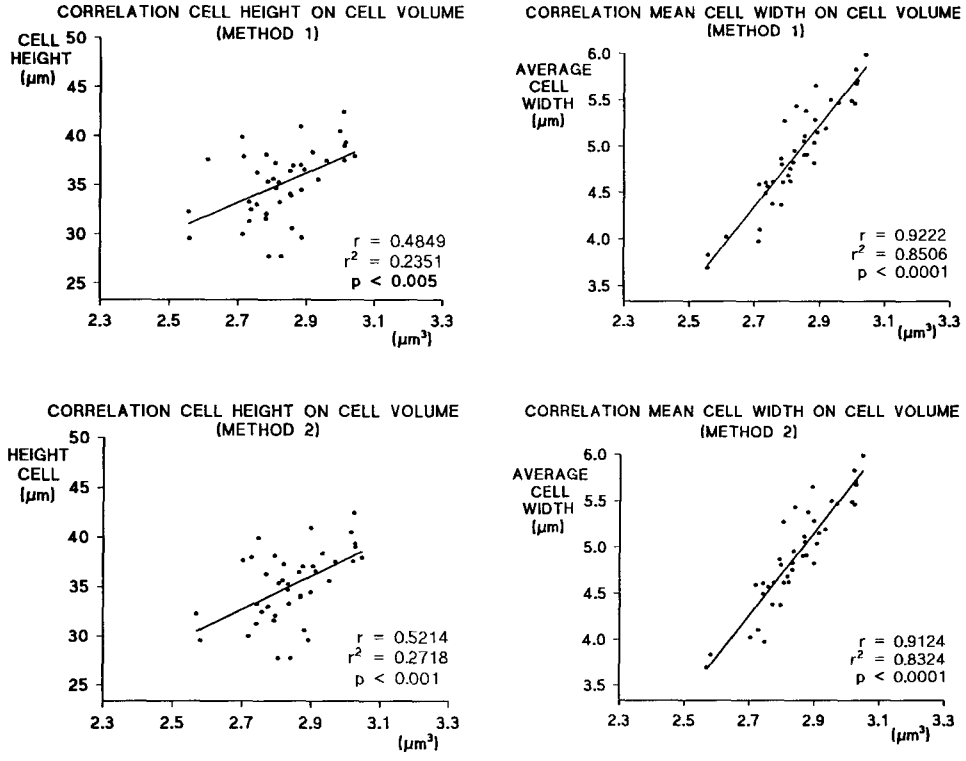

Fig. 5. Correlations between cell height (*left hand panels*) and cell width (*right hand panels*) and cell volumes (log-transformed) by method 1 (*upper panels*) and method 2 (*lower panels*). Cell width shows a good relationship to (log) cell volume, but this is not seen with cell heights where <30% samples show any direct correlation with their corresponding cell volumes

Table 3. Summary of morphometric data for mucosa

	Disease-controls ($n=21$)	Untreated gluten-sensitivity ($n=20$)	*P* value
Surface epithelial volume (V_{SE})[a] ($\times 10^6$ μm^3)	2.3 (1.5–3.6)	0.4 (0.2–0.6)	<0.001
Number of enterocytes[a] (N_{SE}) Method 1:	3040 (1960–4720)	610 (325–1140)	<0.001
Method 2:	2930 (1935–4435)	590 (320–1100)	<0.001
Method 3 (i):	527 (274–1014)	520 (225–1190)	NS
(ii):	494 (313–776)	440 (270–710)	NS
Based on intraepithelial lymphocyte profile density count %:	1464 (870–2470)	312 (160–615)	<0.001
Number of intraepithelial lymphocytes[a] ($N_{V,SE}$):	350 (275–450)	190 (150–240)	0.001

[a] Denotes logarithmic tranformation
NS, not significant
Figures in parentheses are 95% confidence limits

gluten-sensitive mucosae 600 [610 (325–1140) and 590 (320–1100)], based on methods 1 and 2 for calculating cell volumes (Table 3). The group differences indicate a reduction of four fifths (80%) in the average surface complement of enterocytes in gluten-sensitive flat mucosae compared with control tissues, and compared with a similar reduction (82%) in surface epithelial volumes. From the total number of IEL ($N_{V,SE}$) and their mean volume (V_{NU}), the total lymphoid volume ($N_{V,SE} \times V_{NU}$ μm^3) as a proportion of V_{SE} could be determined. This is 0.65 (0.5–1.3) for controls, and 2.5 (1.1–4.1) for gluten-sensitive mucosae ($P<0.001$; Table 4), i.e. IEL occupancy of surface epithelium in latter is approximately increased by a factor of 4.

Given V_{SE}, the absolute number of enterocytes contained ($N_{V,SE}$), and IEL density counts per specimen, the volume of epithelium occupied by a single IEL could also be calculated. This is ~6500 μm^3, equivalent to ~8 enterocytes, for control IEL and ~1900 μm^3, equivalent to ~3 enterocytes, for coeliac IEL. In terms of percentages, these figures become 12 (10–16) IEL/100 enterocytes in the former, and 32 (27–37) IEL/100 enterocytes in the latter ($P<0.01$), compared with conventional profile density count (IEL per 100 enterocytes) of 24 (11–53) and 61 (31–122) respectively; evidently, the latter ratio method (Ferguson and Murray 1971) overestimates the true relationship of IEL to enterocytes by a factor of 2.

Furthermore if IEL/100 enterocyte profile count densities are used to derive data, further discrepancies arise. For example, enterocyte volumes are almost two-fold higher than those calculated by methods 1 and 2, and

Table 4. Summary of morphometric data for intra-epithelial lymphocytes

	Disease-controls ($n=21$)	Untreated gluten-sensitivity ($n=20$)	P value
Mean nuclear profile diameter ($D_{N,IEL}$; μm):	4.9 (4.2–5.6)	5.2 (4.6–5.9)	<0.02
Mean nuclear volume ($V_{N,IEL}$)[a] (μm³:)	63 (41–99)	74 (53–105)	<0.02
Percentage of the epithelium (V_{SE}) occupied by IEL[a]:	0.65 (0.5–1.3)	2.5 (1.1–4.1)	<0.001
Volume epithelium (V_{SE}) occupied by one IEL[a] (μm³):	6450 (2150–19300)	1880 (945–3750)	<0.001
Number of enterocytes per single IEL[a]:	8 (7–11)	3 (2–4)	<0.001
Number (actual) of IEL per 100 epithelial cells[a] (%):	12 (10–16)	32 (27–37)	<0.001
Number (relative) of IEL nuclear profiles/100 enterocyte nuclear profiles[a]	24 (11–53)	61 (31–122)	<0.001

[a] Denotes logarithmic transformation
Figures in parentheses are 95% confidence limits

the number of enterocytes contained within V_{SE} are 1464 (870–2470; controls) and 312 (160–615; untreated gluten sensitivity) which is approximately one-half of those calculated above (Table 3).

Discussion

The application of computerised morphometry to the study and investigation of intestinal structure should be expected to yield additional data and understanding of the information previously obtained by other less direct techniques. In our earlier work we established parameters for volumes of lamina propria (Dhesi et al. 1984), of surface (Niazi et al. 1984) and of crypt epithelium (Marsh and Hinde 1986), and for the typical cell populations contained therein. All such measurements were controlled by reference to a constant test area of muscularis mucosae, thereby permitting the direct comparison of mucosae of varying degrees of transition from normal to enteropathic "flat".

The results of the present study extend those previous analyses, and provide additional new information on intestinal cell and nuclear volumetric dimensions, as well as further observations concerning the relationship between surface (villous) enterocytes and the lymphocytes partitioned on that side of the basal lamina (the IEL). Our data clearly show that the volume of enterocytes on the surface of flat mucosae from patients with untreated gluten sensitivity is significantly smaller than that of control enterocytes. Thus it is possible to conclude from these data that the reduced height profile that characterises the surface epithelium in these flat mucosae is a true reflection of smaller cells.

Before proceeding further, how can we be certain that our varied measurements of cell volume are correct? In recent studies of jejunal mucosa (in hamsters), the volume of dispersed enterocytes (V_{ENT}) was determined by electronic counting (MacLeod et al. 1991). In isotonic saline, V_{ENT} was 1390 ± 190 μm³, which is tolerably close to ~800 μm³ (ranges 475–1247 μm³ and 510–1260 μm³ by methods 1 and 2 respectively). Fixation and embedding causes some 15–20% contraction of original hydrated tissues, while dispersed cells may increase in volume once released from cell-cell and cell-matrix connections. Given these approximations then our values, obtained by direct geometrical methodology related to fixed cells, seem reasonably valid.

There is further indirect proof that the above values are the most accurate. The test area (10^4 μm²) upon which our computerised morphometric programme is based is one which, statistically, represents the area overlain by a single villus. It has been shown (Hagemann et al. 1970) that a single villus in murine small intestine is, on average, covered by ~3000 (±400) epithelial cells, a figure which is virtually identical to our own estimation here (Table 3; by methods 1 and 2) which is based both on an entirely different approach and also on enterocyte volumes of approximately 800 μm³.

Values obtained by the volume to area ratio method [Table 2; methods 3 (i) and (ii)] appear to have been somewhat underestimated. This may have resulted from nuclear volumes in gluten-sensitized mucosae being disproportionately increased relative to cell areas, perhaps because of depressed tissue folate levels (Swanson et al. 1966; Hermos et al. 1972; Klipstein et al. 1973; Davidson and Townley 1977). Such a local abnormality, in disturbing ratios between nuclear and cell volumes, would result in coeliac and disease-control enterocyte volumes being virtually identical. Furthermore, because of the marked pleomorphic disposition of nuclear profiles, either of the geometric models employed for determining nuclear volume could be somewhat unrepresentative.

Cell volumes obtained by method 4 were extremely high. These were based on calculations of the number

of enterocytes derived from knowledge of the absolute lymphocyte count ($N_{V,SE}$) (Table 3) and of the IEL/enterocyte profile density (Table 4): hence, total volume of surface epithelium (V_{SE}) divided by calculated number of enterocytes yields an average volume per enterocyte. It should be noted that all derived calculations, in which densities of IEL/100 enterocytes were used, seemed to be aberrant, including number of enterocytes (Table 3) as well as actual, compared with relative, ratios of IEL to enterocytes (Table 4).

This study has, for the first time, clearly shown that the reduced height profiles of surface enterocytes in untreated gluten sensitivity do reflect a true reduction in cell volumes. Although this may seem a rather obvious conclusion, it is by no means self-evident. This is because epithelial cells can rapidly change shape in response to various external, noxious influences (Table 1), as is also evident from various studies of the resolution of surgically inflicted wounds to epithelia or epidermis. In all such studies (Florey and Harding 1935; McMinn and Mitchell 1954), the viable cells nearest the periphery of the lesion rapidly migrated into the defect, thereby providing a cytoplasmic seal across the defect and its subjacent coagulum of tissue debris. Those cells initially involved in the migration across the defect did so rapidly and became flat, plate-like structures: subsequently with time they progressively became cuboidal, and then columnar in profile, as healing was gradually completed (Florey and Harding 1935; McMinn and Mitchell 1954).

By analogy, it was never entirely certain that the increased rate of crypt cell productivity in gluten sensitivity was always capable of matching the markedly increased rate of surface cell desquamation. In the presence of relative crypt failure, for example, the area of contact of surface cells would increase in order to maintain complete coverage of the basal lamina. This could result in reduced height profiles, although such appearances would not necessarily imply a cell of smaller volume, in comparison with the adjacent cells lining the crypt mouths.

In this regard, we examined the relationship between cell volumes and the corresponding two-dimensional scalars of height and width. It is interesting that although both measures reveal statistically significant correlations with cell volume (both for control and gluten-sensitized mucosae), cell width is a far better predictor of cell volume than cell height. However, the ratio of height to width for each sample of control, and gluten-sensitive cells, was identical suggesting that as the enterocyte becomes smaller, there are parallel reductions both in height and width. The measurement of cell width is less easy than that of height, so it is likely that the latter will continue to be used to monitor cell volume: our data affirm that this still remains a very useful, but not ideal, approach.

We have extended our observations to examine further the volumetric relationships between surface epithelium, and the IEL population. Since the mean diameter of IEL per specimen had been calculated, it was possible to determine mean volumes, and hence the total volume of the IEL pool within surface epithelium (volume occupancy). Our data show an approximate four-fold increase in IEL per unit volume of surface epithelial volume in gluten-sensitive mucosae compared with controls (Table 4), thus confirming their increased density. Note that this increased density occurs (1) despite $\sim 80\%$ reductions in V_{SE} (per $10^4\ \mu m^2$ muscularis mucosae) and in the number of enterocytes which occupy the surface of a flat mucosa (Table 3), and (2) significant reductions in mean IEL population size (Table 3).

The data also permitted an estimation of the true relationship between IEL and enterocytes. In control mucosae there was, on average, one lymphocyte per ten enterocytes, and in flat gluten-sensitive mucosae, one lymphocyte per three enterocytes, leading to values of 13 and 34 IEL/100 enterocytes, respectively (Table 4). These values are less, by a factor of 2, than those obtained by counting profile densities of IEL nuclei/100 enterocyte nuclei – which constitutes the commonest approach to the enumeration of IEL (Ferguson and Murray 1971). Thus, on these grounds, we again reaffirm our previous views (Marsh 1980; Niazi et al. 1984) that this method is unsafe and clearly inaccurate (Guix et al. 1979; Marsh 1980; Niazi et al. 1984; Jenkins et al. 1986; Loft 1991), despite its popularity and widespread usage in monitoring the apparent size of lymphocyte populations both within jejunal (Ferguson and Murray 1971) and rectal (Austin and Dobbins 1988) epithelium.

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