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Mechanisms of adaptation in rat small intestine: regional differences in quantitative morphology during normal growth and experimental hypertrophy

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INTRODUCTION

Adaptations of small intestine to different levels of nutrients and calories include alterations in gross and microscopical morphology (see Karasov & Diamond, 1983 for a review). Changes in cell proliferation rate affect the numbers of absorptive epithelial cells (enterocytes), villous surface area and, perhaps also, the rate at which cells mature as they migrate from crypt base to villous crest (Dowling & Riecken, 1974; van Dongen, Visser, Daems & Galjaard, 1976; Leblond, 1981; Robinson, Dowling & Riecken, 1982; Potten & Loeffler, 1987). In turn, the number and maturity of cells on villi determine the extent and the activity of the striated brush border (Brown, 1962; Pothier & Hugon, 1980). This microvillus–glycocalyx complex then affects the potential for incorporation of membrane-associated hydrolytic enzymes and various transporter molecules (Olsen, Agresti & Lorentzsonn, 1974; Ferraris & Diamond, 1986; Friedman, 1986).

In previous reports, we have described and validated sampling protocols which allow comprehensive descriptions of small intestinal morphology (Mayhew, 1984, 1988; Ross & Mayhew, 1984; Mayhew & Middleton, 1985; Mayhew, Ross & Middleton, 1988). The methods have been used to investigate the ways in which crypts, villi and microvilli vary along the intestine (Mayhew & Middleton, 1985) and to study the effects of a short period of fasting (Ross & Mayhew, 1984, 1985).

In rats and mice, intestinal epithelium exhibits continuous renewal, turnover time being 2–3 days (Leblond, 1981; Cheng & Bjerknes, 1982). If the duration of fasting is less than this, the main morphological response seems to be villous atrophy, attributable to thinning rather than to loss of villous height (Ross & Mayhew, 1984, 1985). Loss of epithelial cell height (Stenling & Helander, 1981) could account for villous thinning. Failure to detect significant alterations in total numbers of microvilli (Mayhew, 1987) would be consistent with the idea that early changes involve loss of cell size and not loss of cell number. However, with longer periods of fasting, mitotic activity within crypts declines due to increased cell cycle times and atrophy is accompanied by hypoplasia (Altmann, 1972; Aldewachi, Wright, Appleton & Watson, 1975).

Structural adaptations similar to those just described characterise hypoplasia induced by hypophysectomy (Bastie *et al.* 1982). In order to examine the mechanisms which obtain in other proliferative states, we have collected material to represent normal growth and intestinal hyperplasia. Though hyperplasia occurs during lactation (Boyne, Fell & Robb, 1966), after bowel resection (Dowling, 1974) and in other

situations (Karasov & Diamond, 1983), we have chosen to induce hyperplasia by means of the diabetogenic agent, streptozotocin. Here we focus attention on differences in composition of the bowel wall and on the physical dimensions of its principal ingredients.

MATERIALS AND METHODS

Experimental groups and tissue preparation

Thirty male Sprague–Dawley rats were maintained in plastic cages on a 14 hour–10 hour light–dark cycle and fed on a standard pellet diet with free access to drinking water. At the onset of the experiment, when animals were about 11 weeks old and 400–500 g in body weight, they were divided into three groups of equal size and approximately equal mean body weight (460 g).

Animals in one group (designated 11wC) were killed at 11 weeks of age. Those in a second group (23wD) were made diabetic by an intraperitoneal injection of streptozotocin under ether anaesthesia and at a dosage of 49–67 mg/kg body weight. Animals were considered to be adequately diabetic if their blood glucose levels exceeded 14 mmol/l when estimated 48 hours after streptozotocin injection. The diabetic animals were maintained for 12 weeks after induction and then killed. Rats in the third group (23wC) were also killed at 23 weeks of age to serve as age-matched controls for the diabetics. One 11wC animal was excluded from the study because of malperfusion. Three diabetic animals died after 8 weeks.

Diabetics and their controls were weighed for the last time prior to killing. Blood samples were obtained from each animal by venesection of tail veins. Samples were taken once per week at midday. The results of blood glucose analyses undertaken on these samples are only summarised because they have been provided in greater detail elsewhere (Bhoyrul *et al.* 1988).

In order to minimise the effects of circadian differences, animals were killed under ether anaesthesia between 9.30 a.m. and 12.30 p.m. They were killed by intra-cardiac perfusion of an isotonic saline pre-wash followed by 2·5% glutaraldehyde in Millonig's phosphate buffer (pH 7·2–7·4). Both perfusates were delivered by simple gravity feed and at ambient temperature.

Tissue sampling protocol

After fixation *in situ*, each small intestine (extending from the pylorus to the ileocaecal junction) was freed of attached mesentery and removed. The length was then measured. Subsequently, every intestine was sliced transversely with a razor blade into four segments of roughly equal length. This number was chosen because earlier studies using only three segments failed adequately to resolve regional differences in villous surface areas (Ross & Mayhew, 1985). Each segment was sliced in turn into smaller pieces from which four were selected by lottery. Luminal contents were flushed out with fresh fixative solution before each piece was trimmed to a length of about 2 cm. The four pieces from each segment were immersed in fixative prior to further processing. Later, all were washed in buffer solution, postfixed for 2 hours in 1% phosphate-buffered osmium tetroxide and dehydrated in graded concentrations of ethanol. Pieces of tissue were embedded in Araldite in flat-bottomed dishes in order to keep a check on tissue orientation. In preparation for sectioning, each piece was removed by hacksaw and re-embedded so that cross-sections of intestine could be cut.

One block was chosen at random to represent each intestinal segment. One arbitrarily positioned thin section was cut from each block using either glass knives

(for 11wC and 23wC intestines) or stainless steel blades (for the grossly distended 23wD intestines). All sections were stained with toluidine blue.

Each transverse section (one per segment per rat) was projected on to a sheet of white drawing paper using a Zeiss DL II projection system, care being taken to ensure that images were flat and undistorted. The final magnifications ($\times 39$, $\times 55$ or $\times 72$) were calibrated with the help of a micrometer scale standard. Traces of the outlines of the primary mucosa (running between bases of villi and openings of crypts), villi, boundary between submucosa and muscularis externa and boundary between muscularis externa and serosa were drawn onto the paper with a sharp pencil.

Stereology and morphometry

Full details of methods for estimating tissue volumes and surface areas have been provided elsewhere (Mayhew, 1984; Mayhew & Middleton, 1985). Segmental estimates of the surface areas of the primary mucosa (to be envisaged as the internal aspect of a long, smooth tube) and of villi were derived by intersection counting methods. For this purpose, tracings of intestinal cross-sections were analysed with a test grid of squares of edge lengths 1 cm (11wC and 23wC groups) or 3 cm (group 23wD). Grids were superimposed on tracings so as to be independent random in both orientation and location (Mayhew, 1983).

The volumes of villi, submucosa and muscularis externa per segment were estimated separately by point counting, using the 1 cm grid superimposed on tracings. The volume of crypts in each segment was derived by multiplying submucosal volume in that segment by the volume density of crypts in the submucosa. Crypt volume densities were estimated directly on the projected cross-sections by point counting.

Crypt volume estimations served two purposes. First, the rate of crypt cell production partly depends on cell number (see Al-Mukhtar, Polak, Bloom & Wright, 1982) which is proportional to crypt volume. Secondly, segmental values of villous surface area, divided by corresponding crypt volumes, provide measures of the relationships between the non-proliferative and proliferative compartments (Mayhew & Middleton, 1985).

Mean villous height per segment was estimated as the mean of five measurements made with a ruler. In an attempt to minimise under-estimation error due to angle and position of section planes, only the tallest profiles in each segment were measured. The arithmetic mean thickness of the muscularis externa was estimated from at least two sites (along the short axis if the bowel cross-section was elliptical in outline) and at most four (along random radii if the cross-section was near-circular).

Total volumes and surface areas per animal were calculated simply by adding the appropriate segmental values. On the reasonable assumption that no changes in numbers of villi per intestine occurred (Clarke, 1972; Forrester, 1972), we attempted to quantify villous 'shape' by an approach described previously (Ross & Mayhew, 1984). This involved computing a dimensionless coefficient, $(S^{1.5})/V$, where S is the total volume of all villi in one animal. If villi in different experimental groups of rats were to change their sizes but not their shapes or numbers, the coefficient would not alter.

Estimates of the perimeter length of the primary mucosa were obtained by dividing total surface area of primary mucosa by intestinal length (Mayhew & Middleton, 1985). Perimeter length is related to mean intestinal 'diameter'.

Table 1. *Body weight, total length and mean perimeter of small bowels in* 11 *weeks old* (11*wC*) *and* 23 *weeks old* (23*wC*) *normal animals and in* 23 *weeks old streptozotocin-diabetics* (23*wD*)

Values are group means (S.E.M.).

Variable	11wC ($n = 9$)	23wC ($n = 10$)	23wD ($n = 7$)
Body weight (g)	467 (10·8)	648 (12·3)*	316 (17·4)†
Intestinal length (cm)	100 (1·93)	114 (2·49)*	121 (3·32)
Intestinal perimeter (mm)	12·6 (0·22)	11·1 (0·29)*	17·7 (0·98)†

* Significantly different from younger rats (11wC).
† Significantly different from age-matched controls (23wC).

Statistical analyses

Initially, all values were calculated per intestinal segment and combined to obtain group means and standard errors (S.E.M.) Regional differences within intestines were examined using Page's L-test for related samples (Miller, 1975). To resolve segmental differences from those due to the effects of growth (11wC *versus* 23wC rats) or diabetes (23wC *versus* 23wD rats), two-way analyses of variance (Sokal & Rohlf, 1981) were undertaken. The interaction term generated by these tests served to indicate whether or not growth or diabetes influenced various intestinal segments to differing degrees.

Values per animal (i.e. per whole intestine) were employed also to determine group means and S.E.Ms. To test for differences in body weights, intestinal lengths, intestinal perimeters and villous shape coefficients between groups, unpaired Student's t tests were applied.

All these calculations were handled using a BBC microcomputer running BASIC programs written for the purpose. A probability level of $P > 0{\cdot}05$ was considered to indicate no statistically significant effect/difference.

RESULTS

In both groups of normal animals, weekly blood glucose levels varied from 3·1–7·0 mmol/l with most values falling between 4·0 and 6·0 mmol/l. By way of contrast, weekly mean blood glucose values in the diabetics invariably exceeded 25 mmol/l. The lowest figure estimated for any individual animal in this group was 16·9 mmol/l.

Body weight, intestinal length, intestinal perimeter

The results are summarised in Table 1.

During the 12 week experimental period, 23wC rats gained a significant amount of weight. The gain in body weight amounted to about 2·2 g *per diem*. Over the same period, 23wD rats lost weight at an average rate of 1·8 g *per diem*. At the end of the experiment, diabetic animals weighed approximately half as much as age-matched controls.

Normal intestines increased in length but decreased in perimeter with time. Intestinal length increased by 14% whilst the mean perimeter of the primary mucosa decreased in about the same proportion (12%). The apparent increase in intestinal length in 23wD *versus* 23wC rats was not statistically significant. However, the mean perimeter of diabetic intestines was substantially greater than that in age-matched controls (about 60%).

Table 2. *Microscopical dimensions in different segments of small bowels during a 12 week period of normal growth*

Values are group means (S.E.M.).

Variable		Intestinal segment (numbered from pylorus) 1	2	3	4
		(A) Surface areas (cm^2)			
Primary mucosa	11wC	29 (1·12)	30 (1·11)	36 (1·72)	30 (1·58)
	23wC	28 (1·78)	33 (1·58)	36 (2·12)	29 (1·73)
Villi	11wC	181 (18·7)	172 (14·0)	134 (10·5)	104 (2·58)
	23wC	182 (8·69)	213 (14·3)	158 (6·20)	127 (6·97)
		(B) Volumes (mm^3)			
Villi	11wC	995 (140)	898 (50·0)	666 (45·3)	438 (37·9)
	23wC	896 (73·3)	992 (64·7)	726 (50·1)	471 (35·1)
Submucosa	11wC	876 (85·7)	769 (39·1)	809 (50·7)	672 (35·1)
	23wC	697 (37·8)	623 (41·6)	649 (38·9)	598 (40·2)
Crypts	11wC	262 (22·6)	222 (20·9)	278 (24·7)	231 (20·2)
	23wC	190 (10·8)	167 (14·2)	178 (17·2)	169 (13·4)
Muscularis externa	11wC	242 (27·8)	242 (15·1)	234 (17·6)	218 (15·9)
	23wC	187 (14·8)	208 (15·0)	212 (11·9)	158 (12·1)
		(C) Distances (μm)			
Height of villi	11wC	542 (19·1)	466 (19·2)	312 (13·9)	269 (27·4)
	23wC	599 (17·1)	514 (17·2)	370 (25·3)	290 (12·5)
Muscularis thickness	11wC	57 (4·4)	60 (5·2)	63 (3·4)	62 (4·9)
	23wC	52 (4·7)	44 (2·8)	44 (3·1)	52 (3·6)
		(D) Ratio (mm^2/mm^3)			
S(v)/V(cr)	11wC	71 (6.49)	87 (14·1)	50 (3·86)	48 (5·05)
	23wC	97 (4·33)	134 (14·6)	95 (7·69)	78 (5·58)

Note that 'submucosa' includes the crypt compartment.
S(v)/V(cr) is villous surface/crypt volume.

Intestinal morphometry

Our findings for the normal animals are compared in Table 2 and for diabetics *versus* age-matched controls in Table 3.

(*a*) *Effects of position along the bowel*

Significant regional differences were detected in all groups for the surface area of primary mucosa and mean villous height. The heights of villi tended to be smallest in the terminal ileum.

In both normal groups, the differences in the heights of villi were associated with gradients of villous surface area, the peak of which appeared to shift from proximal and towards mid-intestinal segments during the growth period. The volumes of the villi varied in a similar pattern but submucosal volumes tended to be greatest most proximally. No regional fluctuation in crypt volume was detected. Therefore, the ratios of villous surface/crypt volume tended to be highest near mid-intestine and to decline progressively thereafter.

Whilst there was no significant variation in volume or thickness of muscularis externa along the intestines of younger animals, these dimensions did vary in older individuals.

Table 3. *Microscopical dimensions in different segments of small bowels from streptozotocin-diabetics and their age-matched controls*

Values are group means (S.E.M.).

Variable		Intestinal segment (numbered from pylorus) 1	2	3	4
		(A) Surface areas (cm^2)			
Primary mucosa	23wC	28 (1·78)	33 (1·58)	36 (2·12)	29 (1·73)
	23wD	43 (3·38)	48 (1·20)	62 (5·76)	62 (8·47)
Villi	23wC	182 (8·69)	213 (14·3)	158 (6·20)	127 (6·97)
	23wD	337 (52·8)	344 (21·9)	356 (39·9)	319 (50·7)
		(B) Volumes (mm^3)			
Villi	23wC	896 (73·3)	992 (64·7)	726 (50·1)	471 (35·1)
	23wD	1515 (262)	1427 (102)	1634 (210)	1251 (184)
Submucosa	23wC	697 (37·8)	623 (41·6)	649 (38·9)	598 (40·2)
	23wD	1138 (87·1)	1071 (731)	1315 (170)	1168 (102)
Crypts	23wC	190 (10·8)	167 (14·2)	178 (17·2)	169 (13·4)
	23wD	376 (25·5)	303 (20·6)	419 (50·2)	322 (48·2)
Muscularis externa	23wC	187 (14·8)	208 (15·0)	212 (11·9)	158 (12·1)
	23wD	335 (22·1)	412 (25·7)	481 (57·8)	470 (55·1)
		(C) Distances (μm)			
Height of villi	23wC	599 (17·1)	514 (17·2)	370 (25·3)	290 (12·5)
	23wD	622 (46·4)	541 (35·1)	533 (31·3)	415 (46·8)
Muscularis thickness	23wC	52 (4·75)	44 (2·76)	44 (3·12)	52 (3·60)
	23wD	49 (4·77)	47 (5·22)	51 (3·06)	52 (7·34)
		(D) Ratio (mm^2/mm^3)			
S(v)/V(cr)	23wC	97 (4·33)	134 (14·6)	95 (7·69)	78 (5·58)
	23wD	89 (11·4)	116 (10·6)	88 (9·07)	102 (13·0)

Note that 'submucosa' includes the crypt compartment.
S(v)/V(cr) is villous surface/crypt volume.

The main differences witnessed in diabetic rats were the lack of regional differences in villous surface area (despite the gradient of villous height), villous volume, volume of submucosa, thickness of muscularis externa (despite the gradient of muscularis volume) and in the villous surface/crypt volume ratio. However, crypt volume varied regionally, tending to be greater in the distal segments of the bowel.

(*b*) *Effects of normal growth*

Intestines in older rats possessed a much more extensive villous surface but smaller volumes of muscularis externa and submucosa. At least part of the loss of submucosal volume could be attributed to a reduced volume of crypts and that of muscularis volume to a decrease in overall thickness of the two smooth muscle layers. The more extensive surface of villi was partly attributable to the presence of taller villi.

No significant growth changes were detected for the surface area of primary mucosa or for the volume of villi. The mucosal surface area was maintained because the older intestines, though narrower, were also longer.

No significant interaction terms were generated. This suggests that the effects of growth, where present, influenced all regions of small intestine in essentially the same way.

Table 4. *Total surface areas, volumes and villous shape coefficients per bowel during normal growth and streptozotocin-diabetes*

Values are group means (S.E.M.).

Variable	11wC ($n = 9$)	23wC ($n = 10$)	23wD ($n = 7$)
Primary surface (cm^2)	126 (4·09)	126 (5·46)	215 (14·7)†
Villous surface (cm^2)	591 (29·2)	680 (22·9)*	1356 (93·5)†
Villous volume (mm^3)	2996 (178)	3084 (157)	5827 (378)†
Submucosa volume (mm^3)	3126 (153)	2566 (120)*	4693 (290)†
Crypt volume (mm^3)	991 (53·4)	704 (38·8)*	1412 (106)†
Muscularis volume (mm^3)	936 (50·2)	765 (32·9)*	1699 (123)†
Shape coefficient	4847 (257)	5802 (237)*	8590 (586)†
Villous surface/ crypt volume (mm^2/mm^3)	60 (2·96)	98 (3·98)*	97 (5·83)

* Significantly different from younger rats.
† Significantly different from age-matched controls.

Anisomorphic growth changes in the dimensions of villi were found, villous shape coefficients being larger in older animals (Table 4).

(*c*) *Effects of diabetes*

The intestines of diabetic animals had considerably greater amounts of all principal components: mucosa, submucosa (including crypts) and muscularis externa (Table 3). An increase in the volume of the muscularis externa was not detected by estimating thickness. Both the primary mucosal and villous surface areas were larger, the latter being partly due to an increase in villous height.

Expansion of the crypt compartment was commensurate with the change in villous surface area. Consequently, the villous surface/crypt volume ratio did not alter.

Except for the heights of villi, we did not find any significant interaction terms. The implication is that experimental diabetes exerted a preferential impact on the villi in distal segments. Some support for this interpretation comes from the results of the Page's trend-tests applied to regional data. These showed that differences in villous volume and surface area existed in age-matched controls but not in the diabetics.

The dimensionless villous shape coefficient in diabetics was greater than that in corresponding controls (Table 4). Thus, changes in the sizes of villi also involved changes in shape.

(*d*) *Values per animal*

These are summarised in Table 4. The main differences during normal growth were as follows. Over the 12 week period, the surface area of villi expanded by 15%, whilst the volume of submucosa diminished by 18%, that of crypts by 29% and that of smooth muscle layers by 18%. Differential effects on the surfaces and volumes of villi resulted in a 63% larger villous surface/crypt volume ratio and a 20% larger villous shape coefficient.

The changes associated with experimental diabetes were not those which could be expected with growth alone. In comparison with their age-matched controls, diabetics had substantially greater amounts of primary mucosal surface area (up 71%) and villous surface (almost doubled). Tissue compartments were also much more voluminous: villous volume increased by 89%, submucosal volume by 83%, crypt volume by 100% and the volume of the muscularis externa by 122%. Again, villi altered anisomorphically, coming to possess a greater surface area than that which could be expected for a change in volume but not in shape. However, the ratio of villous surface/crypt volume remained remarkably constant in both groups.

DISCUSSION

The present investigation has shown that quantitative changes in the composition of the intestinal wall and the dimensions of its mucosal surface occur during normal growth and in experimental diabetes. It has also confirmed the existence of regional differences along the small intestine. The efficiency of the underlying stereological methods and the benefits which they offer to studies on the functional morphology of small intestine have been discussed elsewhere (Mayhew, 1984, 1988; Mayhew & Middleton, 1985; Ross & Mayhew, 1984, 1985).

Present results highlight the disadvantages of adopting linear dimensions instead of surfaces or volumes. In an earlier study on the effects of fasting, we found no significant alteration of bowel length or circumference yet the surface area of mucosa (the product of length and circumference) decreased by 16% (Ross & Mayhew, 1985). In another investigation (Ross & Mayhew, 1984), we noted that villous height was a poor variable for monitoring the effects of fasting on villous surface area. Height is equally inappropriate in other situations (Hromádková & Skála, 1968; Clarke, 1974; Lorenz-Meyer, Köhn & Riecken, 1976). Now it appears that thickness of muscularis externa must be put in the same class. It does not provide an infallible way of monitoring changes in muscle mass; after all, this depends not only on thickness but also on bowel circumference at the submucosa/muscularis boundary and on bowel length. Therefore, conclusions based on the use of this variable (e.g. Diani, Gerritsen, Stromsta & Murray, 1976) should be drawn with caution.

The regional differences in mucosal architecture noted correlate well with gradients of transport rates (Karasov & Diamond, 1983) and are essentially the same as those described by us and by other workers. In earlier studies on normal rats, we found regional differences in surface area of primary mucosa and in surface, volume, height and shape of villi but failed to find any segmental variation in crypt volume (Mayhew & Middleton, 1985; Ross & Mayhew, 1984, 1985). Qualitative examination of intact villi has revealed that regional differences in shape do exist (Clarke, 1970). Quantitative studies have shown gradients of intestinal circumference, villous height, relative surface area of villi, villous epithelial mass and ratio of villous/crypt epithelial mass but no regional variation in crypt size, epithelial mass, mitotic index or colchicine index (Fisher & Parsons, 1950; Boyne *et al.* 1966; Altmann & Enesco, 1967; Hromádková & Skála, 1968; Diamond *et al.* 1984). The list of regional differences in structure along the small intestine can now be extended to include the volume of submucosa and, at least in older rats, the volume of muscularis externa.

In the case of morphological changes occurring with growth or age, direct comparisons between the present and previous findings must be drawn with care. Amongst other uncertainties, actual ages may not be available for comparison. Using body weight as an alternative to age is likely to be unreliable unless such factors as sex, strain, diet and breeding conditions are taken into consideration. Confining comparisons to older (rather than developing or juvenile) animals, we find agreement with earlier studies for changes in villous shape (Baker, Mathan & Cherian, 1963; Miller *et al.* 1969) and it is possible that the longer and narrower intestines of older animals reflect this. In contrast, discrepancies do exist for alterations in intestinal length, intestinal circumference, relative villous surface area and crypt content (Clarke, 1977; Penzes & Skála, 1977). Similar inconsistencies have been reported for nutrient transport rates (see Karasov & Diamond, 1983). Clearly, the effects of growth and age on intestinal structure and function require further investigation.

The increase of villous surface/crypt volume during growth was due to villous

hypertrophy and crypt atrophy. It is possible that the difference reflects a slower growth rate in older animals. Cell proliferation in the intestines of younger rats contributes to epithelial growth as well as renewal: that in older animals contributes solely to renewal (Altmann & Enesco, 1967). Therefore, intestines in older rats, including experimental diabetics, may conform to a different 'steady state'. However, Altmann & Enesco (1967) reported a decline in the ratio of villous/crypt epithelial cell numbers and this is difficult to reconcile with the changes in surface/volume ratio seen here.

The streptozotocin-diabetic animals used in this study demonstrated all the symptoms of severe, untreated diabetes, viz., loss of body weight, abnormally high blood glucose levels and premature death. In addition, they were hyperphagic and polydipsic, eating and drinking 2–3 times more than end-control animals (unpublished observation). The increased volumes of mucosa, submucosa and muscularis externa support the increases in intestinal and mucosal mass reported by others (Jervis & Levin, 1966; Schedl & Wilson, 1971; Lal & Schedl, 1974) though in the present study they were associated with disproportionately greater changes in intestinal diameter than length. The changes in volume/mass emphasise the need to express functional data such as nutrient transport rates on a suitable basis so as to avoid making misleading interpretations of their biological significance (Schedl & Wilson, 1971; Karasov & Diamond, 1983).

Larger volumes of both proliferative and non-proliferative compartments are consistent with increases in the lengths of crypts and heights of villi (Jervis & Levin, 1966; Stenling, Hägg & Falkmer, 1984). However, our unpublished findings suggest that the increase in crypt volume can be explained entirely by an increase in diameter and not by an increase in length. The higher villous shape coefficient implies that the changes in villous dimensions are anisomorphic, height altering to a different extent than base length and/or thickness. Hypertrophy and/or hyperplasia must account for these differences in villous size and shape because the number of villi per intestine is probably constant (Clarke, 1970, 1972; Forrester, 1972).

It is known that intestinal water content (Schedl & Wilson, 1971; Nakabou, Okita, Takano & Hagihira, 1974) and protein:DNA ratio (Nakabou *et al.* 1974) may be higher in diabetes and there is morphometric evidence that epithelial cells at the tips of villi are taller, at least in the jejunum (Stenling *et al.* 1984). Other morphometric investigations have reported increased numbers of cell profiles per villous profile (Keelan, Walker & Thomson, 1985).

Though compatible with hyperplasia, such data are certainly not conclusive because of the added possibility of changes in cell and villus size and shape. However, DNA content is greater in diabetic intestines (Nakabou *et al.* 1974) which also exhibit greater crypt activity and decreased turnover time of villous epithelial cells (Miller, Hanson, Schedl & Osborne, 1977). Therefore, it is likely that the greater volumes of villi and crypts arise by hyperplasia combined with hypertrophy. The more voluminous crypt compartment must also contribute to the greater volume of submucosa but the mechanism by which muscularis externa volume expands needs further enquiry. In the spontaneously diabetic hamster (Diani *et al.* 1976), vascular lesions and vasodilation occur within the submucosa and the muscularis externa becomes thinner (but not necessarily less voluminous!) with fibrous connective tissue infiltration.

With the single exception of villous height, the structural changes found here during diabetes appeared not to be localised preferentially in particular segments. The greater impact on villous height distally supports morphometric findings of alterations in the jejunum but not in the duodenum (Stenling *et al.* 1984), kinetic findings of increased

crypt cell proliferation in the ileum but not the jejunum (Miller *et al.* 1977) and functional data concerning enzyme activities in the jejunum *versus* the ileum (Olsen *et al.* 1974). Though no significant interaction terms were demonstrable in this study, our results still allow the possibility that diabetes produced greater increases in villous surface area in more terminal segments. Thus, segmental differences in villous surface area were significant in age-matched control animals but not in diabetics. An alternative explanation for these morphological and functional observations is that there is an upper limit to the amount of hypertrophy which intestinal segments can undergo and that proximal segments are closer to this limit.

Quantitative differences in mucosal architecture in diabetes are associated with enhanced absorption of water and nutrients (Olsen & Rosenberg, 1970; Schedl & Wilson, 1971; Caspary, 1973; Lal & Schedl, 1974; Nakabou, Ishikawa, Misaki & Hagihira, 1980; Keelan *et al.* 1985). The proximate causes of all these adaptations are presently unknown but possible candidates include hormonal, vascular and luminal factors. Loss of glucose in the urine may lead to compensatory changes in intestinal structure and functional capacity. Hyperphagia associated with various other conditions, e.g. lactation, bowel resection, reduced ambient temperatures and hypothalamic lesions, leads to mucosal changes similar to those in diabetes (Boyne *et al.* 1966; Genyk, 1971; Karasov & Diamond, 1983). Hormonal disturbances, e.g. of glucagon as well as insulin levels, occur in diabetics. Intestinal epithelial cells bear surface receptors for insulin (Forgue-Lafitte, Marescot, Chamblier & Rosselin, 1980) and injection of insulin can prevent the elevated glucose absorption seen in diabetes (Caspary, 1973).

The enormously expanded mucosal volume and surface area reported here undoubtedly contribute to the longer term enhancement of nutrient absorption seen in experimental diabetes. However, they cannot explain the more immediate increases seen within hours of onset and before any anatomical changes are demonstrable (Olsen & Rosenberg, 1970; Schedl & Wilson, 1971; Caspary, 1973; Lal & Schedl, 1974; Olsen & Korsmo, 1975; Nakabou *et al.* 1980).

SUMMARY

The gross and microscopical dimensions of small intestines from three groups of rats were investigated by morphometric (mainly stereological) methods. The groups were chosen to represent relatively 'steady state' situations: normal growth (over a 12 week period) and intestinal hyperplasia due to streptozotocin-diabetes of 12 weeks duration. Four intestinal segments were sampled along each intestine.

For normal groups, no interaction effects were found, suggesting that growth affected all regions of the small intestine in the same way. Older rats were heavier and their intestines were longer and narrower. In addition, villous surface area was more extensive and the villi differed in shape. Volumes of crypts, submucosa and muscularis externa were all reduced.

Diabetic animals weighed less than age-matched controls and their intestines were wider but not significantly longer. All surface areas and volumes were increased substantially. However, hypertrophy of the muscularis externa was not detected by measuring muscularis thickness. Villi altered their shape. At least for villous height, the effects of diabetes were greater in terminal segments.

These findings are discussed in the context of the reported effects of age and experimental hyperplasia (including diabetes) on intestinal architecture and behaviour.

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