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Effects of fasting on mucosal dimensions in the duodenum, jejunum and ileum of the rat*

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INTRODUCTION

It has been reported that the absorption of sugars and amino acids from the small intestine can increase, decrease or remain unaltered after fasting or after restricted food intake (reviewed by Karasov & Diamond, 1983). At least part of the confusion produced by these contradictory interpretations arises because of a failure to standardise the morphological base – intestinal weight, length or surface area – to which transport rates are referred (Diamond *et al.* 1984). Since absorption takes place across the apical surfaces of villous epithelial cells, and since proximodistal gradients of regional transport rates are accompanied by gradients of villous surface area (Fisher & Parsons, 1949, 1950; Diamond *et al.* 1984), it is preferable to express physiological data in terms of surface areas rather than in terms of weight or length. Intestinal weight decreases following reduced food intake (Steiner, Bourges, Freedman & Gray, 1968; Debnam & Levin, 1975; Lipscomb & Sharp, 1982) and it has been shown that transport per mg of intestinal weight can increase in experimental animals even though transport per cm^2 of villous surface area declines (Diamond *et al.* 1984). Unfortunately, little information is available on villous surface area in fasted animals or even in controls (Mayhew & Middleton, 1985).

In this paper, advantage is taken of recent developments in intestinal morphometry (Mayhew, 1984) to investigate the effects of fasting on mucosal dimensions in the duodenum, jejunum and ileum of rats. Special attention is given to the surface areas and volumes of villi.

MATERIALS AND METHODS

Animals

Twelve adult female rats of the black and white hooded Lister strain, weighing 267–308 g, were employed. They were assigned to two groups of six animals so that group mean body weights were approximately equal. Both groups were housed in plastic cages under normal laboratory conditions for a period of two days to permit acclimatisation to their environment. During this time, both groups were maintained on a 12 hour–12 hour light–dark cycle and allowed free access to standard pellet diet and drinking water.

At noon on Day 0 of the experiment, all animals were reweighed. For the next two days, one group was deprived of food but allowed water *ad libitum*. The other group served as controls and continued to receive food and water as required.

At noon on Day 2 of the experiment, all animals were weighed once more.

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Tissue preparation and sampling

After their last weighing, animals were anaesthetised with ether and killed by intracardiac perfusion with isotonic saline prewash followed by 2·5 % glutaraldehyde in 0·1 M Millonig's phosphate buffer, pH 7·3, at room temperature. After fixation, the entire small intestine (from pylorus to ileocaecal junction) was freed of mesentery and removed. Its length was then measured.

The intestine from each rat was sliced transversely into three segments of roughly equal length. Each segment was sliced further into three shorter pieces from which one piece per segment was chosen at random. Luminal contents were flushed away with fresh fixative solution and the tissue trimmed to a length of about 2 cm. From such 2 cm lengths, small pieces of tissue representing three segments per rat (designated as duodenum, jejunum and ileum for descriptive convenience) were placed in fresh fixative for one more hour. They were then washed in buffer solution, post-fixed for 2 hours in 1 % buffered osmium tetroxide and dehydrated in graded ethanols. All pieces were embedded in Araldite resin in flat bottomed containers. Later, they were removed and affixed to dummy Araldite blocks so that intestinal segments could be sectioned transversely.

One arbitrarily located semithin section, c. 1 μm thick, was cut from each block of tissue and stained with toluidine blue. One or more micrographs were recorded per section and printed to a final linear magnification of $\times 56$ as determined with the aid of a calibration standard. Where necessary, photomontages of complete transverse sections through intestinal segments were constructed from these final prints.

Stereological procedures

Full details of the principles for estimating pertinent morphometric variables are given elsewhere (Mayhew, 1984; Mayhew & Middleton, 1985). Segmental estimates of the circumference of the primary mucosa (defined as the boundary running between the bases of villi and the openings of crypts of Lieberkühn), the amplification of the primary mucosal surface by villi and the surface areas of primary mucosa and of villi were derived by intersection counting methods. Volumes of villi per segment were obtained by point counting. Numbers of test line intersections and numbers of test points were counted using a quadratic lattice of spacing 1 cm, this spacing being equivalent to a distance of 0·179 mm on the actual specimens. The lattice was superimposed on each micrograph in turn so as to be independent in both position and orientation.

The heights of villi on sections were measured with a ruler. Four profiles per intestinal transverse section were measured in order to estimate the height of the average villus in each segment.

Subsequently, segmental estimates of mucosal surface areas and villous volumes were employed to calculate total values per animal and to determine average villous surface amplification factors for the small intestine as a whole.

Statistics

Initially all values were estimated per intestinal segment and combined to calculate group means and standard errors (S.E.M.). Later, two-way analyses of variance (Bishop, 1971) were performed in order to isolate segmental effects from fasting effects. The interaction values obtained by applying these tests served to indicate

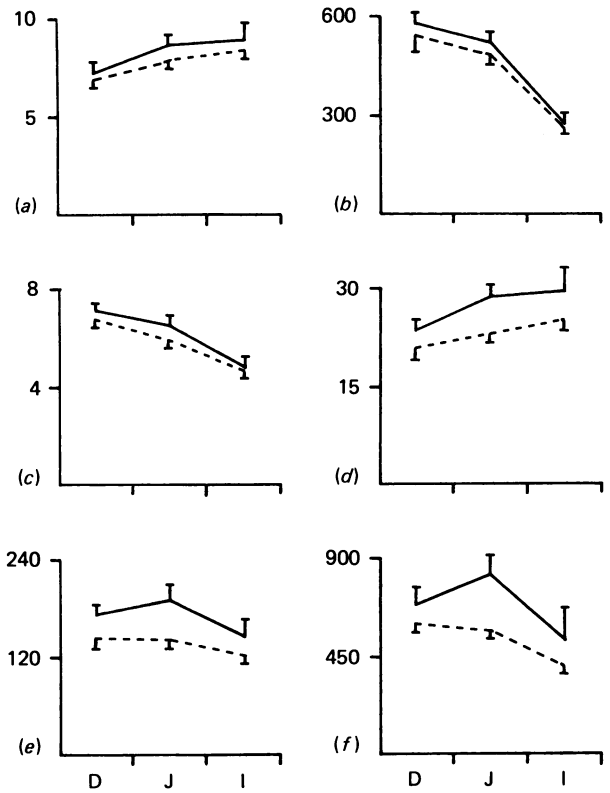

Fig. 1(*a–f*). Gradients of mucosal architecture revealed in duodenum (D), jejunum (J) and ileum (I). Variables illustrated are: (*a*) the circumference of the primary mucosa in mm; (*b*) mean villous height in μm; (*c*) villous amplification factor in cm^0; (*d*) primary mucosal surface area in cm^2; (*e*) villous surface area in cm^2 and (*f*) volume of villi in mm^3. Solid lines, control animals; broken lines, fasted animals. Bars denote S.E.M. ranges.

whether or not fasting had any significant selective influence on the duodenum, jejunum or ileum.

Values per animal were also used to determine group means and S.E.M. values. As measures of variability between animals, observed coefficients of variation were calculated and then corrected to true estimates of the coefficients of variation by isolating the variances due to within-animals (i.e. between-segment) differences (Mayhew & Middleton, 1985).

To test for differences between body weights at Day 0 and Day 2, paired Student's *t*-tests were performed on both control and fasted groups. For other comparisons, unpaired *t*-tests were applied.

All calculations were handled using a Hewlett-Packard HP85 personal computer running BASIC programs.

RESULTS

Findings are illustrated in Figure 1*a–f* and summarised in Tables 1–3.

On Day 0, group mean body weights in the control and fasted groups of animals were 277 and 281 g respectively (Table 1). By Day 2, control animals had gained a significant amount of weight (mean $\pm$ S.E.M.: 11 $\pm$ 2·8 g) whilst fasted animals had

Table 1. *Differences in body weights of control and fasted rats between Day* 0 *and Day* 2 *of the experiment. Values are group means* ± S.E.M. *in g*

Group	Day 0	Day 2	Difference	Paired *t* value*	*P* value
Control	277 ± 7·40	288 ± 5·52	11 ± 2·84	3·8	< 0·02
Fasted	281 ± 5·54	260 ± 5·45	21 ± 1·60	13·0	< 0·001
Unpaired *t***	0·4	3·6			
P value	ns	< 0·01			

ns denotes not significant; * degrees of freedom, 5; ** degrees of freedom, 10.

Table 2. *Summary of two-way analyses of variance performed on the segmental estimates illustrated in Figure* 1. *Values are variance ratios*

Variable	Segment effects (D.F. 2, 30)	Treatment effects (D.F. 1, 30)	Interaction (D.F. 2, 30)
Circumference	5·5 ($P < 0·01$)	1·7 (ns)	0·1 (ns)
Villous height	51·3 ($P < 0·001$)	0·9 (ns)	0·6 (ns)
Villous amplification	32·6 ($P < 0·001$)	2·2 (ns)	0·5 (ns)
Surface of primary mucosa	4·4 ($P < 0·05$)	10·3 ($P < 0·01$)	0·2 (ns)
Surface of villi	3·1 (ns)	10·8 ($P < 0·01$)	0·5 (ns)
Volume of villi	5·0 ($P < 0·05$)	6·6 ($P < 0·05$)	0·6 (ns)

ns denotes not significant; D.F. denotes degrees of freedom numerator, denominator.

lost a significant amount of weight (21 ± 1·6 g). Thus, fasted animals had lost about 10% of body weight as compared to controls (Table 1).

On Day 2, mean intestinal length in the control group was 100·5 ± 3·52 cm. The apparent 10% shortening observed in fasted rats (90·5 ± 6·73 cm) was not statistically significant (degrees of freedom, 10; $P > 0·10$).

Regional differences in primary mucosal circumference, villous height, villous amplification factor, primary mucosal surface area, villous surface area and villous volume are shown in Figure 1 for both experimental groups. Results of two-way analyses of variance applied to these data are given in Table 2.

Significant differences between segments were established for all variables except the surface area of villi. In both groups of rats, the circumference of the primary mucosa increased towards the terminal ileum and accounted for the similar pattern of increase in primary mucosal surface area. In contrast, the height of the average villus, the volume of villi and the villous amplification factor all declined towards the terminal ileum. Changes in amplification factors were due to regional differences in primary mucosal surface area rather than villous surface area.

Fasting had significant effects on the primary mucosal surface area, villous surface area and villous volume but no significant influence on circumference, villous height or villous amplification factor. In no case was there any significant interaction effect. This implies that fasting had a similar effect on all intestinal segments and did not exert any preferential influence on the duodenum, jejunum or ileum.

Taken together, these findings indicate that the rats responded to fasting by decreases in mucosal surface areas (primary and villous) and villous volumes per segment.

Values per animal are given in Table 3. The main differences between groups were

Table 3. *Intestinal dimensions per animal. Variability between animals within a group is expressed as values of observed and true coefficients of variation (CV), the latter being estimated by subtracting within-animals variances from observed between-animals variances*

Variable	Group mean ±S.E.M.	Observed CV	True CV
Primary mucosal surface, cm^2			
Control	82·6±4·74	14%	3%
Fasted	69·1±3·56	13%	11%
Villous surface, cm^2			
Control	510±42·6	20%	18%
Fasted	403±26·1	16%	15%
Villous volume, mm^3			
Control	2067±243	29%	23%
Fasted	1603±114	17%	13%
Villous amplification, cm^0			
Control	6·1±0·24	10%	0%
Fasted	5·8±0·12	5%	0%

as follows. After fasting, the primary mucosal surface diminished by about 16% (from 83 to 69 cm^2), the surface area due to villi by 21% (510 to 403 cm^2) and the volume of villi by 25% (2067 to 1603 mm^3).

The results further suggest that whilst total surface areas and volumes show real differences between animals (true values of the coefficient of variation 3–23%), villous amplification factors seem to be fixed from one animal to the next (true coefficient of variation, 0%).

DISCUSSION

In this experiment, depriving animals of food for two days led to a significant drop in body weight, amounting to roughly 10% of the control value. At the same time, certain intestinal dimensions were also affected. Apparent decreases in intestinal length and circumference were not statistically significant. Nevertheless, their *combined* effect was to reduce significantly the surface area of the primary mucosa (i.e., the smooth intestinal tube) by 16%. Stenling & Helander (1981) starved rats for 24 hours and found significantly reduced intestinal lengths both before and after fixation but no alteration in circumference, at least for the duodenum. These findings suggest that lengths or circumferences alone are relatively insensitive indicators of the effects of fasting and that it may be preferable in general to monitor experimental effects by estimating *both* variables and then calculating surface areas.

The change in primary mucosal surface area was accompanied by equiproportional decreases in the surface area and volume of villi (about 20%). These alterations were therefore greater than the relative loss of body weight and they affected all three regions of the small intestine examined in this study in the same way. Fasting had no effects on mean heights of villi or on the surface amplification factors due to villi in these three regions.

The gradients of mucosal structure revealed in this report are similar to those described in other investigations (Fisher & Parsons, 1950; Boyne, Fell & Robb, 1966; Altmann & Enesco, 1967; Hromádková & Skála, 1968; Altmann, 1972;

Lipscomb & Sharp, 1982; Diamond *et al.* 1984; Mayhew, 1984; Mayhew & Middleton, 1985). The failure to detect significant regional differences in villous surface area disagrees with an earlier examination of more segments from more animals (Mayhew & Middleton, 1985) and is probably due to the smaller sample sizes (i.e. fewer animals and fewer segments per animal).

Present results confirm that villous height and villous surface amplification factor are powerful estimators for detecting structural differences between intestinal segments. Of the two, villous height has the practical benefit that it is easier to estimate from histological sections. However, these quantities were unsatisfactory discriminators of differences between control and fasted animals. To this extent, these findings support those of Lipscomb & Sharp (1982) who failed to demonstrate changes in heights of villi following a 60% reduction of food intake for 20 days and those of Stenling & Helander (1981) who found no evidence of alterations in villous amplification factors after 24 hours of starvation. In view of these observations, it seems expedient to exercise caution in the choice of morphometric variables when comparing experimental groups of animals.

Though present results are based on smaller samples, control values of 83 cm^2 (primary mucosal surface), 510 cm^2 (villous surface) and 2100 mm^3 (villous volume) agree tolerably well with previous estimates of 96 cm^2, 510 cm^2 and 2850 mm^3 respectively (Mayhew & Middleton, 1985). They also substantiate the earlier findings that whilst absolute values demonstrate real differences between animals, villous amplification factors are constant from one rat to the next. These conclusions can now be extended to include fasted rats.

Many studies have shown that starvation and semistarvation affect body weights. Depending upon species, previous diet, initial body weight and period of restricted food intake, decreases of 10–30% have been reported (Steiner *et al.* 1968; Clarke, 1972; Lipscomb & Sharp, 1982; Diamond *et al.* 1984). The effects on intestinal weight are less clear cut. Prolonged semistarvation apparently has a preferential impact on the ileum (where intestinal weight is reduced approximately in proportion to body weight) and on the thickness of circular and longitudinal components of the duodenal tunica muscularis (Lipscomb & Sharp, 1982). This situation differs from that in starved animals where intestinal weight decreases overall by 53% when body weight drops by only 32% (Steiner *et al.* 1968). Both the mucosa and tunica muscularis are affected. In this study, the loss of villous volume (from 2100 to 1600 mm^3 per rat) affected all regions and was relatively greater than the loss of body weight. Moreover, since villous volume accounts for almost 50% of total tissue volume in control intestines (Mayhew, unpublished observation), alterations in this compartment must make a substantial contribution to any overall reduction in intestinal weight (Steiner *et al.* 1968).

The number of villi stays constant not only through most of the normal lifespan of the rat but also after various experimental treatments (Clarke, 1970, 1972; Forrester, 1972). This implies that mucosal atrophy involves alterations in the size rather than in the number of villi. Since the volume and surface area of villi diminish equiproportionally but villous heights are unchanged, the alterations in villous size must be anisomorphous. This has been confirmed by quantifying dimensionless, shape-dependent coefficients (Ross & Mayhew, 1984). Villi in all regions of the rat small bowel respond to fasting by altering their shapes: they become thinner.

The phenomenon of villous thinning probably reflects changes in intestinal blood flow and in enterocyte size and/or number. On the basis of analyses of total intestinal

and mucosal DNA, RNA, protein and water, Steiner *et al.* (1968) concluded that fasting leads to fewer and smaller cells. Following six days of complete starvation, RNA, protein and water contents all decreased in proportion to the 53 % drop in intestinal weight. These changes were in sharp contrast to the changes in DNA which diminished by only 36 %. The alterations in cell size have been substantiated, to some degree, by morphometry. Stenling & Helander (1981) found significant decreases in the mean heights of the more mature enterocytes at the tips of villi in the duodenum and proximal jejunum of fasted rats. No data were provided for more distal intestinal segments. However, cell heights at the tips and bases of villi in the ileum may also decrease (Ross, unpublished observations).

Morphometric estimates of cell numbers are liable to various types of systematic error (Weibel, 1979) and these may account for some of the apparent discrepancies previously recorded. In this context, it is worth noting that Altmann & Enesco (1967) proposed estimating the numbers of cells per average villus from favourable histological sections passing along the villous height axis. This so-called 'villus size index' has been employed repeatedly to test for differences within and between animals (Altmann & Leblond, 1970; Altmann, 1972; Lipscomb & Sharp, 1982). This index is not only an unnecessarily laborious way of estimating villous height but it may be an unreliable index of relative cellularity. In fact, the index is a measure of the number of *cell profiles* per villous section. As such, it is influenced by changes in cell size and villous height (which have been shown to vary within animals and between animals), by changes in section thickness as well as by changes in real numbers of cells.

Using 5 μm thick paraffin sections through duodenum, jejunum and ileum, Lipscomb & Sharp (1982) failed to find any differences between controls and semistarved rats. In contrast, Altmann (1972), employing 3 μm thick sections, reported progressive decreases in villous size indices after 3, 5 and 7 days of starvation. Whilst the decreases were said to be most marked in the duodenum and to diminish towards the ileum, inadequate statistical information was made available to adjudge the significance of these findings. In the present study, two-way analyses of variance did not reveal any fasting or interaction effects on villous heights. Whilst some of these discrepancies might be due to differing experimental protocols, the inherent unreliability of the 'villus size index' cannot be discounted as a possible contributory factor. The same criticisms and inconsistencies apply to the 'crypt size index' which is estimated in a similar fashion (Altmann & Enesco, 1967; Altmann, 1972; Lipscomb & Sharp, 1982). Clearly, there is a need to apply better quantitative methods in order to estimate numbers of villous cells and numbers of crypt cells.

Starvation and semistarvation do not influence significantly the numbers of crypts per intestine (Clarke, 1972; Lipscomb & Sharp, 1982). Therefore, it is unlikely that the losses of villous surface area and volume can be attributed to changes in crypt/villus ratios. However, changes in crypt size may contribute to the observed decreases in these variables and to the decrease in intestinal weight. Crypt depth in semistarved animals may decrease preferentially within the ileum where it is associated with reduced proliferative activity as measured by tritiated thymidine scintillation counting (Lipscomb & Sharp, 1982). The selective effect on ileal crypt size seems to be supported by the results of Altmann (1972) but, again, inadequate statistical data preclude the drawing of firm conclusions. The reduction in proliferative activity may not be confined to the ileum since numbers of mitotic figures per crypt section are *generally* lower after starvation. Altmann (1972) concluded that a

lengthening of cell cycle time might be the most likely explanation for the decreases in the mitotic pool. Indeed, the hypoproliferative response to fasting now appears to be mediated solely by increases in cell cycle times in both rats and mice (Wiebecke, Heybowitz, Lohrs & Eder, 1969; Aldewachi, Wright, Appleton & Watson, 1975). After fasting rats for 4 days, Aldewachi *et al.* (1975) found that the length of the cell cycle increased from a control value of 10·4 hours to one of 14·7 hours. Moreover, sixteen hours after refeeding, the cell cycle time was reduced to 6·5 hours.

These findings suggest that starvation results in a hypoproliferation within intestinal crypts. This response leads to decreases in the sizes of both the proliferative and non-proliferative compartments of the small bowel but not to changes in the numbers of crypts and villi. The changes which affect villi make them thinner but not shorter. As a consequence, total surface areas and volumes of villi per intestine diminish equiproportionally. Since there is no evidence for any preferential impact on any given region, systemic factors would seem to be involved in regulating these changes. This does not exclude the possibility that regional factors maintain pre-existing gradients of villous height and amplification factors (Altmann & Leblond, 1970). As a measure of the rigidity of this control, it may be noted that amplification factors are fixed both within and between control and fasted animals.

In adult rats, the rate of growth of intestinal epithelium is such that most of the cells produced contribute to renewal (Altmann & Enesco, 1967). With a total villous surface area of 510 cm^2 and a whole epithelial turnover time of 60 hours (Cheng & Bjerknes, 1982), control rats must renew roughly 400 cm^2 over the 2 days duration of this experiment. If fasted animals lose villous surface area to the same extent, present results would imply that only 300 cm^2 or 75 % of this surface is renewed because of the increase in cell cycle time.

So far, the effects of fasting on enterocyte microvilli have not been mentioned. Stenling & Helander (1981) observed that microvillous amplification factors did not alter significantly after fasting, at least in the duodenum and proximal jejunum. Changes were noted in the surface area of enterocyte apical plasma membrane expressed per unit of cell volume. This ratio was higher in fasted rats and may reflect the decrease in cell size. The average height of microvilli decreases in fasted hamsters (Misch, Giebel & Faust, 1980), suggesting that in these animals amplification factors may decline (Mayhew & Middleton, 1985). Whether amplification factors remain the same or decrease, the present results suggest that fasted animals have a less extensive surface area due to microvilli.

These findings can be correlated with physiological data. Changes in transport rates for glucose and certain amino acids start to appear in fasted animals at about the same time as anatomical changes. However, the relative decreases in transport rates tend to be greater than those in structural quantities, suggesting that anatomical adaptations offer only a partial explanation of the physiological findings (Karasov & Diamond, 1983).

SUMMARY

Stereological methods were used to investigate the effects of fasting on the duodenal, jejunal and ileal mucosa of rats.

Fasting for two days led to significant reductions in body weight but did not affect significantly intestinal length or primary mucosal circumference. Two-way analyses of variance indicated significant differences between regions in circumference, villous height, villous amplification factor, primary mucosal surface area and villous

volume but no differences in villous surface area. Fasting had significant effects on mucosal surface areas and villous volume but did not alter villous heights or amplification factors. In no case was there any significant interaction effect. Thus, fasting influenced all intestinal regions in the same way.

The surface area of the smooth intestinal tube decreased by 16% whereas villous surface area and volume both decreased by about 20%. Control animals had, on average, 500 cm^2 of villi and 2100 mm^3 of villi. Corresponding figures after fasting were 400 cm^2 and 1600 mm^3 respectively.

Results are discussed in the context of previous findings. The methods described offer the opportunity to express physiological data (transport rates) in terms of a standard reference, i.e., the available absorptive surface area.

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