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The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat

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INTRODUCTION

Recent investigations of epithelial cell turnover and three-dimensional structure of the mucosa of the small intestine were based on the intestinal villus as a unit of architecture (Clarke, 1970*a*, *b*). For long-term studies it would be useful to know whether a single villus is a constant unit of mucosal architecture: does the number of villi change as the animal grows or undergoes experimental manipulations?

Clarke (1967) showed that the number of villi in the duodenum of the chicken was the same three days before hatching as in the adult, and Forrester (1972) has independently concluded that in the rat the number of villi does not alter substantially as the animal grows.

The number of villi and of crypts per unit of 'serosal area' can be counted in the longitudinally opened intestine; the present paper describes how the 'serosal area' may be measured, and multiplied by the population density of villi and of crypts to give estimates of the total number of villi and of crypts in the small intestine. The effect of age and of five days' deprivation of food upon these estimates will be described and discussed. The preliminary findings of this investigation have already been published (Clarke, 1971).

MATERIALS AND METHODS

A total of 33 rats were examined, in six groups of five or six rats. Five groups of rats, of mean weights 28, 111, 161, 290 and 428 g, had had free access to food. A further group of six rats, of mean initial weight 166 g, had a mean weight of 120 g after five days' deprivation of solid food.

Male albino Wistar rats were obtained from A. Tuck and Son, Rayleigh, Essex, or from the Joint Animal Breeding Unit, University of Nottingham, Sutton Bonington; the youngest group of animals was born and reared in the Laboratory's animal house, where all the animals were kept for at least one week, with free access to pellets of Diet 41B and tap water, until death by exposure to chloroform vapour. Fasted animals were kept in cages with wire-mesh floors; they had free access to tap water, but were deprived of solid food for five days before death. After weighing the animal, the whole intestine was removed, and the stomach cannulated. An aqueous solution of 0·9 % sodium chloride at room temperature was flushed through the intestine at a pressure sufficient to dislodge food residues; this was followed by a mixture of absolute ethanol (3 parts) and glacial acetic acid (1 part). After ligating

the colon, the small intestine was distended with fixative to a pressure of 33 cm of fixative. The stomach was then ligated, and the small intestine immersed in the same fixative mixture for 24 hours.

The small intestine was transferred to 70 % ethanol, carefully separated from the stomach and caecum and mesentery, and slit longitudinally along the mesenteric attachment. The whole small intestine was hydrated, subjected to the Feulgen reaction (Wimber & Lamerton, 1963: hydrolysis in N/1 HCl at 60 °C for 12 minutes) and laid out flat, serosa uppermost, in 45 % acetic acid in a shallow Perspex trough. It was covered with a series of microscope slides (3 in × 1 in) laid end to end, to keep it flat and to improve the optics for subsequent photography.

The trough was placed above a strip of recording paper (NS6, Ilford), and a 'contact print' of the intestine was made by exposure to a green light, situated more than one metre above the intestine. Experiments with regular shapes of known area showed that the enlargement due to parallax was less then 1 % of area. The recording paper was developed for two minutes in Phentrace (Ilford) at 22 °C, rinsed in 2 % acetic acid, fixed in Ilfofix (Ilford), washed in tap water, and allowed to dry in air for at least 12 hours. The width of the strip of paper was measured, and the ends cut square to a measured length. This rectangular strip of paper (of known area) was weighed to an accuracy of 1 mg. The image of the intestine was cut from the paper, and weighed in turn. The area of the image bears the same relationship to the measured area of the rectangular strip as does its weight to that of the strip. This area of the opened flat intestine is described as 'serosal area'. Duplicate measurements of the area of images obtained in this way from the same piece of intestine showed a difference of less than 1 %. The weight of exposed and unexposed strips of paper, of identical size, differed by less than 1 %.

Meanwhile the trough and intestine were placed, without disturbing the intestine, on the stage of a microscope, the ends of the trough being supported. Thirteen sites in each intestine were examined, the position of each site being defined as its distance from the pylorus, expressed as a percentage of the length of the whole small intestine. The sites examined were: 1, 5, 10, 20, 30, ... 80, 90, 95, 99. Both crypts and villi can be seen in such a preparation, and each may be sharply focused in turn. Photographs were taken, midway between the mesenteric attachment and the anti-mesenteric border, of crypts at a linear magnification of × 20, and villi at linear magnifications of × 10 or × 6, on 35 mm Pan F film (Ilford). Photographic enlargements of the negatives were made to × 100, × 50 and × 30 respectively, the final magnifications being checked against photographs of a stage micrometer slide. For the crypts one count was made, on the × 100 photographic enlargement of each site, of the number of crypts in a square of side 10 cm, giving an estimate of the number of crypts in 1 mm^2 of 'serosal area' of intestine. For the villi, one or more counts were made, on the × 50 or × 30 photographic enlargements of each site, of the number of villi in a square of side 10 cm, giving an estimate of the number of villi in 4 or 11·1 mm^2 respectively of 'serosal area'. The number of counts of villi performed at each site was the same for a given intestine, and was such that the total scanned area for the thirteen sites exceeded 1 % of the 'serosal area' of that intestine. From the figures for crypts and villi per unit area, the crypt/villus ratio was calculated.

The product of the number of villi per unit area and the 'serosal area' gave an

estimate of the total villi in the small intestine. This calculation was performed in two ways: initially, the intestine was split transversely into thirteen regions, with a sampled site at the centre of each, and the number of villi calculated for each region by multiplying its area (determined by weighing) by its density of villi. These numbers were summed to give the total villi in the small intestine. This total was compared with that reached more simply by assuming the population density at each site to be a sample of that region, weighting the population density according to the *length* (not the area) of each region, summing the population densities and multiplying by the total 'serosal area'. These procedures gave results which differed by less than 2 %. This identity is due to the strong resemblance of the intestine to a cylinder; the diameter (when distended at the stated pressure) is almost constant along the small intestine, except for a slight postpyloric dilatation, and a precaecal narrowing; thus the length of each region was approximately proportional to its 'serosal area'. The second, simpler procedure was followed as a routine, to calculate the number of villi, and the number of crypts, per intestine. Duplicate measurements of serosal area and density of villi on the same intestine gave values for the total number of villi which differed by less than 2 %.

RESULTS

Fed rats

Values for these rats are denoted by closed symbols in Figs. 1 and 3. The measurements of 'serosal area' are shown in Fig. 1A. It should be pointed out that the opened intestine does not lie completely flat, because the mesenteric border is shorter than the anti-mesenteric, like a cycle inner tube. Calculation shows that this causes little error in the measurement of 'serosal area', provided that the intestine is kept reasonably flat; the weight of a microscope slide is sufficient for this task. The 'serosal area' is not of great biological significance; it is merely the area of the intestine of rats of different weight, when distended at a constant pressure.

Fig. 1B shows the number of crypts in the small intestine at each weight. The pattern is similar to that for the 'serosal area'; this confirms an impression from casual observation that the diameter of crypts remains relatively constant throughout life, except in the young rat, where they are both narrower and shorter than in the adult. Crypts probably grow by longitudinal fission; when the mucosa is dissected to release individual crypts (Wimber & Lamerton, 1963) the majority of crypts have the shape shown in Fig. 2(*a*). Occasionally one sees the shapes portrayed in (*b*) and (*d*); (*c*) is rare, so presumably the 'unzipping' occurs at some speed. I have never seen appearance (*e*), and conclude that growth of new crypts takes place by growth and fission of existing crypts from the bottom upwards, rather than *de novo* from the top downwards. This inference is supported by the observation that the bases of all crypts are in close apposition to the muscularis mucosae, a situation which would not obtain if crypts grew down from the crypt/villus junction.

Fig. 1C shows the number of villi in the small intestine at each weight. The number is already at the adult level in the youngest rats examined (21 days old), and continues at this level, with a slight decline in old age. The value at 420 g is significantly different from the 161 g value ($P < 0{\cdot}001$; Student's 't' test).

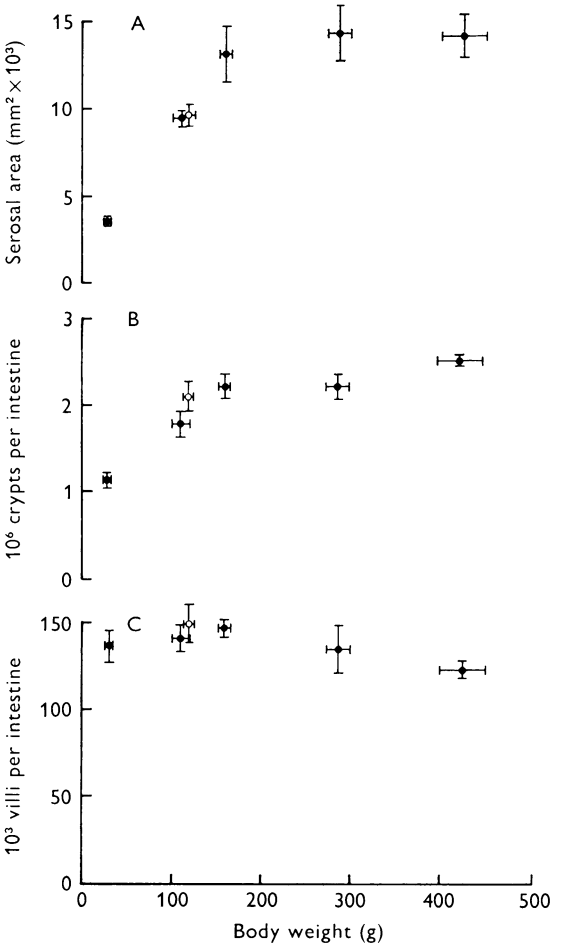

Fig. 1. Relation between small intestine and body weight of rats. Abscissa: body weight in g. ●, fed rats; ○, rats deprived of solid food for five days. Bar represents ±1 s.d. Ordinate: A, 'serosal area' of intestine; B, number of crypts of Lieberkühn in intestine; C, number of villi in intestine.

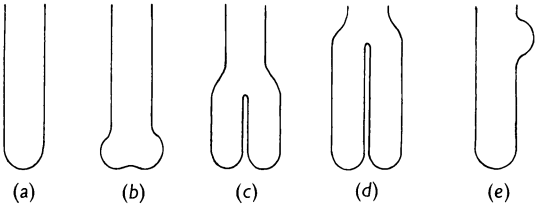

Fig. 2. Diagrammatic representation of shapes of crypt seen on dissection. (*a*) Usual appearance; (*b*), (*d*) seen occasionally; (*c*) very rare; (*e*) not seen.

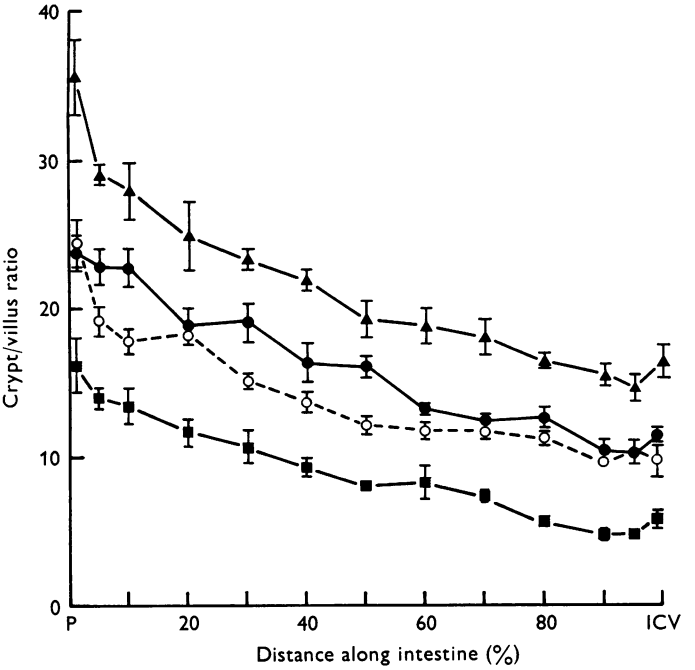

Fig. 3. Relation between crypt/villus ratio and site in the small intestine. Abscissa = distance from the pylorus, expressed as a percentage of the length of the whole small intestine; P = pylorus, ICV = ileo-caecal valve. Ordinate = crypt/villus ratio. ▲ = 438 g group of fed rats; ● = 161 g group of fed rats; ■ = 28 g group of fed rats; ○ = 120 g group of rats after five days' deprivation of solid food. Bar represents ±1 standard error of mean.

Fig. 3 shows the crypt/villus ratios plotted against distance along the intestine for the youngest, middle and oldest groups. Values for the other two groups of fed rats fall intermediate between these, but have been omitted for clarity. The general rise in crypt/villus ratio with age is to be expected from consideration of the numbers of crypts and villi at each weight; these results show that the rise is evenly distributed throughout the length of the intestine.

Starved rats

Values for these rats are denoted by open circles in Figs. 1 and 3, and should be compared with the closed circles representing the 161 g group of animals. Five days' deprivation of food leads to a decline in the serosal area (Fig. 1A) to a value consistent with the weight of the animals. The number of crypts (Fig. 1B) also falls, but not to the same extent; the crypts become smaller, and appear to be spaced out. The difference between the fed and starved values for crypts per intestine is not statistically significant.

The number of villi shows a slight, but not statistically significant, increase after 5 days' food deprivation (Fig. 1C). The combination of this apparent increase and the slight decrease in the number of crypts leads to a fall in the crypt/villus ratio (Fig. 3), which is significant at the 5% level (Student's '*t*' test) at seven of the thirteen sites. In another group of rats starved for five days (R. M. Clarke, un-

published observations), the fall in crypt/villus ratio was less marked; the importance of the fall demonstrated here is doubtful.

DISCUSSION

Forrester (1972) used a similar sampling technique, but inspected his specimens from the mucosal surface; fusion of leaf-shaped villi makes it difficult to distinguish individual villi in the duodenum, and Forrester's results are for the small intestine below the ligament of Treitz, about 10 % from the pylorus. If values for the number of villi in the first 10 % are subtracted from the total in this investigation, then figures similar to Forrester's are obtained; the close agreement between these two independent investigations of the same phenomenon should increase confidence in the results. Forrester's method does not enable the number of crypts to be ascertained, but it may well be better for examining the number of villi in very young animals, where the villi are so slender that they have insufficient optical density for ready detection by the transillumination method used in this investigation. As in Forrester's study, the reproducibility of the results on one intestine suggests that the variation between animals is real.

The constant number of villi in the small intestine of the rat was foreshadowed by a similar finding in the chicken (Clarke, 1967) and by the observations of Miller *et al.* (1969), which were consistent with earlier findings (Baker, Mathan & Cherian, 1963) that, in the proximal small intestine, leaf-shaped villi fuse to form ridges as the rat ages. Viewed from the serosal aspect, it is possible to separate such ridges into their component villi, and the latter were counted in this investigation. In spite of this precaution it was possible that the decline in the number of villi in old rats might have been due to this fusion, but if this were so, then old rats should be found to exhibit a differential decrease in the proximal small intestine. No such decrease was found, implying the reality of the decline with advancing age.

It has been known for years that starvation results in atrophy of the intestine; it is now clear that this atrophy is, in part, due to a decrease in the size of the functional units (villi), rather than to a decrease in their number; likewise hypertrophy, whether from force-feeding or intestinal resection, is the result of an increase in size, but not in number, of the functional units (Forrester, 1972).

Apart from the intrinsic interest of the constancy of the intestinal villus, the finding has considerable significance in that the villus may be regarded, certainly in a statistical sense (and probably in a physical sense also), as a relatively fixed unit of mucosal architecture over a considerable part of the life span of the rat. Estimates of, for example, number of crypts per villus, cell production rate per villus and cell shedding rate per villus acquire quantitative meaning in absolute terms, enabling more precise and relevant observations to be made of the effects of experimental and pathological conditions on mucosal architecture and epithelial renewal in the small intestine.

SUMMARY

Estimates were made of the number of villi and of crypts per unit area at thirteen sites in the small intestine in five groups of fed rats of different ages, and in a group of rats deprived of solid food for five days. Estimates were also made of the total 'serosal area' of the small intestine. The product of these estimates is the total number of villi and of crypts in the small intestine.

The number of villi remained constant in fed and starved rats, except for the oldest fed rats, which had fewer villi. The number of crypts increased with the size of the intestine in the fed rats, and fell slightly in the starved rats. The ratio of crypts to villi rose with age throughout the small intestine of the fed rats, and fell slightly in the starved rats, compared with their fed controls. The importance of the constancy of the intestinal villus is emphasized.

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REFERENCES

BAKER, S. J., MATHAN, V. I. & CHERIAN, V. (1963). The nature of the villi in the small intestine of the rat. *Lancet* i, 860.

CLARKE, R. M. (1967). On the constancy of the number of villi in the duodenum of the post-embryonic domestic fowl. *Journal of Embryology and Experimental Morphology* **17**, 131–138.

CLARKE, R. M. (1970*a*). Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat. *Journal of Anatomy* **107**, 519–529.

CLARKE, R. M. (1970*b*). A new method of measuring the rate of shedding of epithelial cells from the intestinal villus of the rat. *Gut* **11**, 1015–1019.

CLARKE, R. M. (1971). Does the number of villi grow with the rat? *Journal of Anatomy* **109**, 352.

FORRESTER, J. M. (1972). The number of villi in the rat's jejunum and ileum: effect of normal growth partial enterectomy and tube feeding. *Journal of Anatomy* **111**, 283–291.

MILLER, D. S., RAHMAN, M. A., TANNER, R., MATHAN, V. I. & BAKER, S. J. (1969). The vascular architecture of the different forms of small intestinal villi in the rat (*Rattus norvegicus*). *Scandinavian Journal of Gastroenterology* **4**, 477–482.

WIMBER, D. R. & LAMERTON, L. F. (1963). Cell population studies in the intestine of continuously irradiated rats. *Radiation Research* **18**, 137–146.