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Mucosal architecture and epithelial cell production rate in the small intestine of the albino rat

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INTRODUCTION

Recent work has brought about a considerable advance in the understanding of the three-dimensional structure of the intestinal mucosa. Holmes, Hourihane & Booth (1961) pointed out the advantage of examining the mucosal surface with a dissecting microscope, and Creamer and his colleagues (e.g. Loehry & Creamer, 1966) have taken this approach a stage further with the introduction of the autolysis technique, which enables one to examine *post-mortem* the shape of the basement membrane on which the epithelial cells rested.

There has been less progress in obtaining information about the rate of cell production in the crypts of Lieberkühn, largely due to continued reliance on the examination of routine histological sections, and technical innovation is urgently needed here. The relationship between mucosal architecture and cell production rate will not be clarified until both can be examined accurately in the same specimen.

It is the purpose of this paper to show how the two can be correlated by a new combination of existing methods.

The basic technique is that of Wimber & Lamerton (1963). The Feulgen reaction is performed on fixed tissue in bulk, followed by treatment in 45 % acetic acid. This renders the tissue translucent, and friable enough for micro-dissection, makes the nuclei visible, and causes very little shrinkage. The mucosal architecture can then be examined with incident illumination, or the relative densities of villi and crypts can be seen with transmitted light. Whole villi with their crypts may be dissected free for measurements of crypt depth and villus height, and individual crypts can, with practice, be dissected free. If the experimental animal has been subjected to colchicine-blockade before death, the number of colchicine-metaphases per crypt can be counted, if a squash be made by compressing the crypt under a cover-glass. The use of various durations of colchicine-blockade allows an estimate of the frequency of entry of cells into division. This is equal to the rate of cell production per crypt (assuming that for each division *one* cell will migrate out of the crypt). If the number of crypts per villus is known for any given region, then the cell production rate per villus can be computed for that region. In this way the relationship between cell production rate and height of villus will be revealed.

By making reasonable assumptions about the relationship between the height of a villus and the number of epithelial cells clothing it, one can proceed to calculate the

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expected relationship between villus transit times in different regions of the small intestine. Published measurements of these will form a guide to the validity of the assumptions made in the argument.

MATERIALS AND METHODS

Male albino Wistar rats, weight range 143–183 g, were obtained from A. Tuck and Son, Rayleigh, Essex, and kept in the Laboratory's animal house for at least 1 week before use, at a temperature of 21 °C, and illuminated for 9 h per day (0830–1730 h). They were fed pellets of diet 41 B (Oxoid) *ad lib.*, and tap water *ad lib.*, until death between 13.30 and 15.30 h. Three groups of animals were used: one group of 12 was injected intraperitoneally with Colcemid (Ciba) 1·3 mg/kg body weight in Water for Injection B.P. $2\frac{1}{2}$ h before death; the second group of six was similarly injected $\frac{1}{2}$ h before death, and the third group of five comprised normal, uninjected animals.

The small intestine was dissected out, freed from mesentery, and washed out with 0·9 % sodium chloride solution at room temperature. The length of the small intestine was measured, the distal end tied off, and the intestine distended with a mixture of absolute ethanol (3 parts) and glacial acetic acid (1 part), 0·15 ml/cm of intestine (Dowling & Booth, 1967). The upper end was then tied off, and the intestine immersed in the same fixative for 24 h. Drainage holes were cut in the intestinal wall, and the lumen was flushed out with 75 % ethanol. The intestine was then stored in 75 % ethanol until examined.

The small intestine was slit longitudinally along the mesenteric border, and transferred successively to 50 % ethanol (15 min) and water (15 min). It was then stained in bulk by the Feulgen reaction; hydrolysis was carried out in N/1 hydrochloric acid at 60 °C for exactly 5 min; after washing in water, the tissue was transferred to Schiff's reagent for at least 1 h. After further washing in water, the intestine was laid out flat, without stretching, serosa uppermost, in a shallow perspex trough. It was mounted in 45 % acetic acid and covered with a strip of thin, transparent polythene sheet.

Thirteen sites were examined in each of nine intestines (three from each group). The position of a site was defined according to its distance from the pylorus, expressed as a percentage of the total length of the small intestine. The sites examined were: 1, 5, 10, 20, 30, . . ., 80, 90, 95, 99.

The trough containing the intestine was supported on the stage of a Zeiss Photomicroscope. Photographs were taken of the crypts at each site at a magnification of × 20, and of the villi at a magnification of × 10. Occasionally, the use of a green filter improved contrast. The 35 mm negatives thus obtained were enlarged × 5 exactly, to give total linear magnifications on the prints of × 100 and × 50 respectively. For each site one count was made of the number of crypts on the photograph in a square of side 10 cm, which is equivalent to the number of crypts per mm^2 of serosa in the specimen. A similar estimate was made for the villi, to give the number of villi in 4 mm^2 of serosa. From these figures, the number of crypts per villus (the crypt/villus ratio) can be calculated for each site.

A square of tissue, of size about 0·5 cm, was taken from each site in 15 individuals (five from each group), and a number of single villi and their attached crypts were dissected free under a dissecting microscope. The crypt-villus junction is usually

easily visible, and, for each specimen, measurements were made on 10 villi of the height of the villi and the depth of the crypts, using a calibrated eyepiece micrometer. The mean for each site for each animal was calculated.

Finally, in the animals in which mitosis had been blocked by Colcemid, 10 individual crypts were dissected from each specimen and transferred to a drop of 45 % acetic acid on a coverslip, which was then inverted onto a microscope slide. Moderate pressure caused sufficient flattening and spreading for counts of colchicine-metaphases in a single crypt to be made.

RESULTS

Anaphases and telophases were seen in the crypts of seven of the $2\frac{1}{2}$ h Colcemid animals, and in one of the $\frac{1}{2}$ h animals, and no further observations were made on these animals, since escape from colchicine blockade causes an underestimate in the rate of cell production.

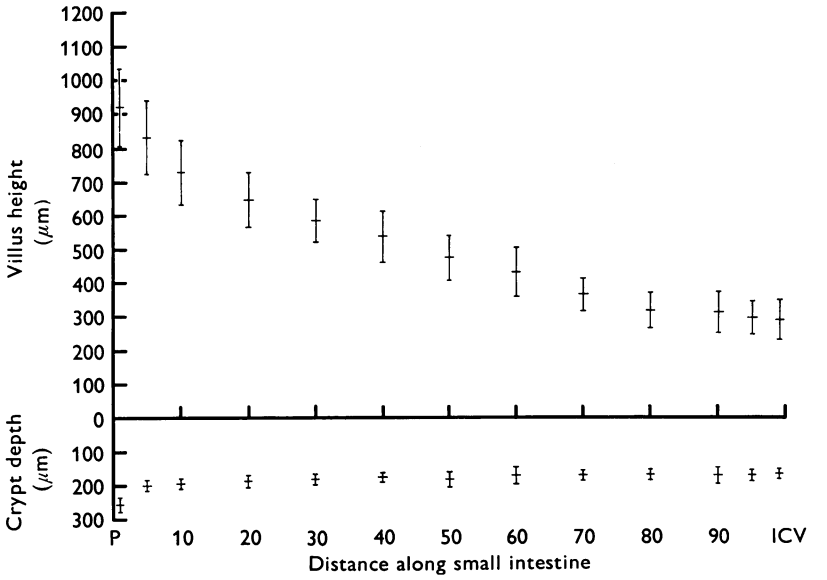


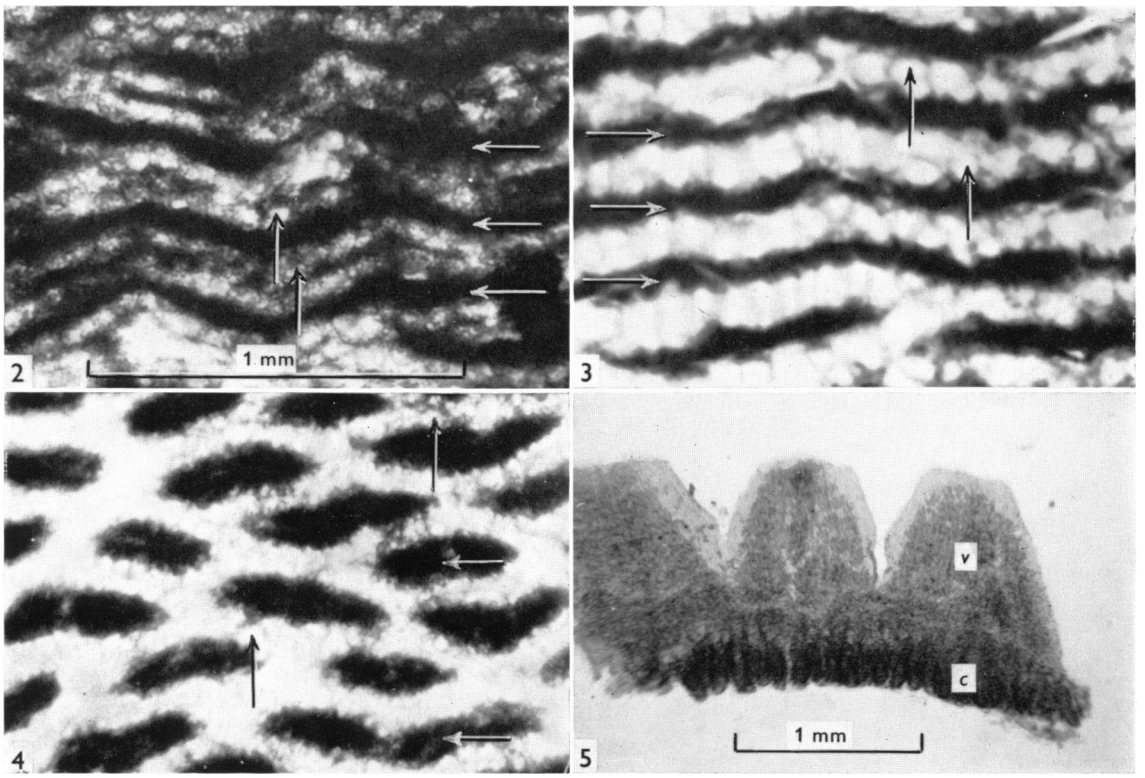
Fig. 1. Abscissa: distance from pylorus expressed as a percentage of length of small intestine (*P* = pylorus, *ICV* = ileocaecal valve); ordinate: above the line, villus height in μm; below the line, crypt depth in μm. Each point represents the mean (±S.D.) of the mean values from 15 animals.

Measurement of villus height and crypt depth

The estimates of villus height and crypt depth in the remaining 15 animals were compared, to see whether there were differences between the three groups of animals. No statistically significant differences were detected (Student's '*t*' test), and the means for all 15 animals were conflated. Fig. 1 shows the villus height and crypt depth for each of the 13 sites (mean ± standard deviation). The height of the villi decreases progressively along the intestine.

Dissecting microscope observations of mucosal architecture

In the duodenum (sites 1 and 5) the villi are often arranged in a zigzag pattern (Fig. 2) reminiscent of the chicken intestine (Clarke, 1967). They are tall, flat in section at the base, and may appear leaf-shaped if examined at the surface. In the proximal jejunum (sites 10 and 20) the villi often form incompletely cleft transverse ridges (Fig. 5), while further distally the villi are separate units, orientated transversely in the intestine, becoming lower, almost mound-like as the terminal ileum is reached (Fig. 4).



Figs. 2–5. Scale line = 1 mm. Figs. 2–4: (same magnification): photomicrographs of pattern of villi (horizontal arrows) viewed from serosal surface in bulk Feulgen-stained preparations. The crypt mouths (vertical arrows) can also be seen, out of focus. Fig. 2: site 5, transverse ridges in slight zigzag pattern; Fig. 3: site 20, transverse ridges; Fig. 4: site 95, individual villi; Fig. 5: photomicrograph of single ridge from site 20, seen *en face*, showing incomplete separation of villi. (*V*, villi; *C*, crypts).

Crypt/villus ratio

This was computed as the number of crypts per mm² of serosa for the site, divided by the number of villi per mm² of serosa for the same site. Fig. 6 shows the mean and standard deviation of the crypt/villus ratio for each site in nine animals. The decline is due principally to an increase in the density of villi in the distal region, and also to a distal reduction in the density of the crypts.

Rate of accumulation of colchicine-metaphases

For each site in each animal the mean of the counts of colchicine-metaphases in 10 crypts was calculated. There is considerable variation both between crypts at the same site in one animal, and between the means for one site in different animals. These variations are probably real, since counts on a single crypt were reproducible (coefficient of variation = 3·9 %), and since counting metaphases from a second set of 10 crypts from the same site in the same animal gave a mean not significantly different from the first (Student's '*t*' test).

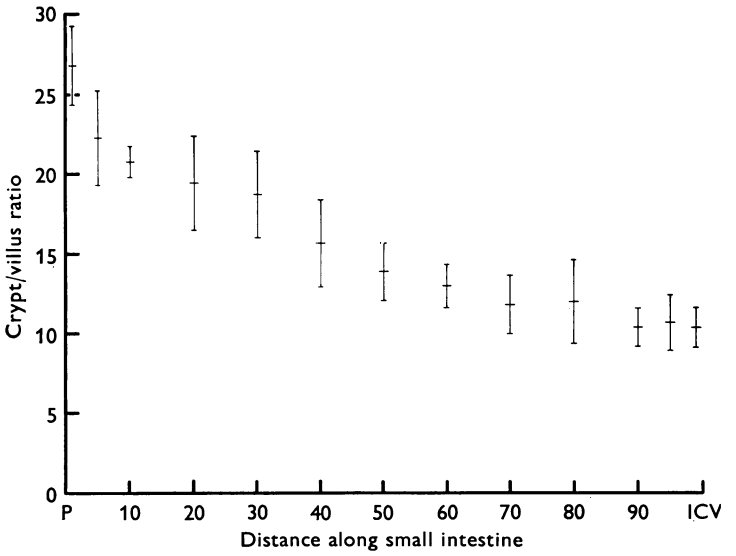


Fig. 6. Abscissa: as Fig. 1; ordinate: number of crypts per villus. Each point represents the mean ±S.D. for 9 animals.

Fig. 7 consists of a series of lines drawn between the ½ h and 2½ h colchicine-metaphase mean counts per crypt for each of the 13 sites. The single vertical bar at each time represents the largest standard deviation (between animals) encountered at that time.

The slope of the line (rate of accumulation of colchicine-metaphases) ranges from 32·8 to 41·5. The apparent delay before the onset of blockade ranges from −0·02 to +0·15 h (−1 to 9 min), with a mean of 0·06 h (4 min).

Fig. 8 shows the rate of accumulation of colchicine-metaphases plotted against distance along the intestine, together with the regression line calculated by the method of least squares. There is a negligible proximo-distal decline of 2·0 colchicine-metaphases/crypt/h, and, to a first approximation, it appears that the number of colchicine-metaphases/crypt/h does not vary significantly along the length of the small intestine.

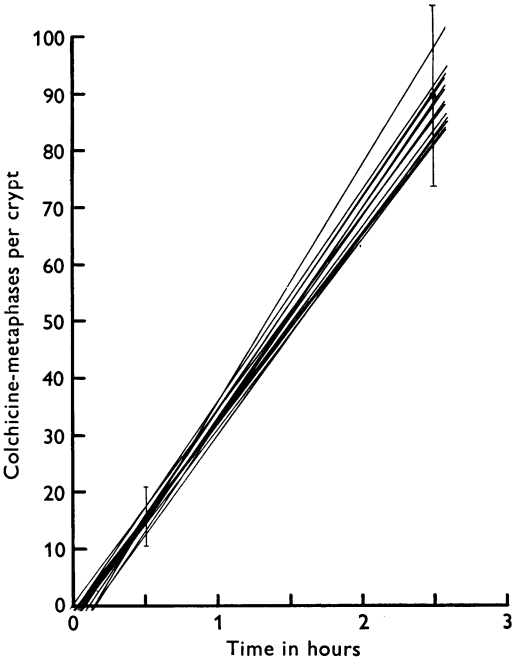


Fig. 7. Abscissa: time elapsing between administration of Colcemid and death; ordinate: number of colchicine-metaphases per crypt. Each point represents the mean of five animals, 10 crypts counted per animal. Vertical bar shows the standard deviation of the set of data with the greatest variance.

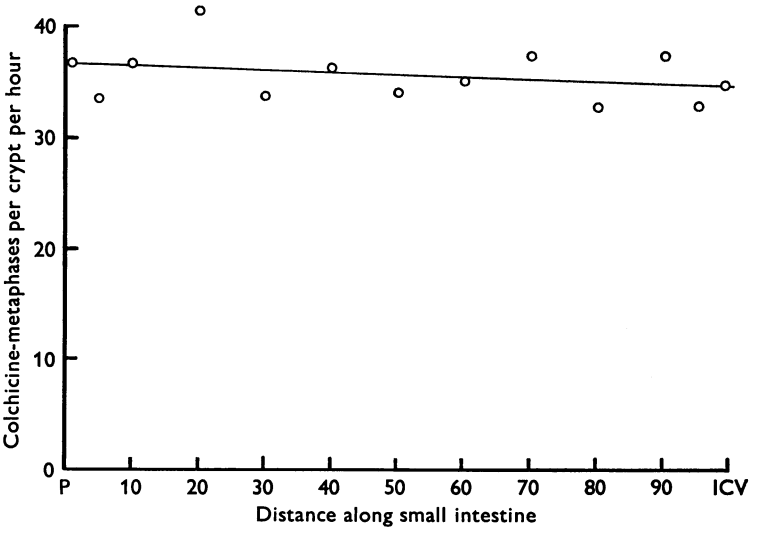


Fig. 8. Abscissa: as Fig. 1; ordinate: colchicine-metaphases per crypt per hour. Each point is calculated from the data shown in Fig. 7. Regression line calculated by the method of least squares.

DISCUSSION

The dissecting microscope observations agree with previous descriptions of the mucosal architecture of the small intestine of the rat (Baker, Mathan & Cherian, 1963), while the measurements of villus height and crypt depth are consistent with the data of Altmann & Enesco (1967), although their absolute measurements are smaller, being made on paraffin-embedded material. It seems likely that the method used in this investigation leads to little or no shrinkage, and thus the measurements given may approximate to those obtaining *in vivo*.

The first innovation is the measurement of the rate of entry of cells into mitosis per whole crypt, rather than per crypt section, or per 100 crypt cells, as has been the custom. Counting objects in histological sections is an exercise whose results require great caution in interpretation, since the apparent population density of the object depends not only upon its real population density, but also upon the thickness of the histological section, the size and shape of the object, and the criteria used to identify it (Abercrombie, 1946; Marrable, 1962; Clarke, 1968). Any method which obviates these corrections, therefore, deserves serious consideration as an alternative to the examination of sections, and the method used in this investigation is especially suitable, since the crypt of Lieberkühn forms a natural unit whose rate of cell-division may be readily assessed. A further advantage of the method is that the time- and labour-consuming stages of paraffin-embedding and sectioning are omitted: the whole gut can be stored indefinitely in 75 % ethanol, yet a specimen from any desired part of it can be available for examination in 2 h from the decision to examine it.

The rate of entry of cells into division per crypt is what is being measured. If (*a*) the cell population of the crypt remains constant in size, and if (*b*) the only route of cell loss from the crypt is by migration on to the villi, then the frequency of entry of cells into division per crypt equals the rate of cell production per crypt. These assumptions require further examination:

(*a*) It is difficult to measure the size of the crypt cell population directly, since the transition from crypt to villus is not abrupt. However, unless there are considerable changes in the packing of cells in the crypts, it is unlikely that there are gross alterations in the size of the crypt cell population unaccompanied by changes in the dimensions of the crypts, especially in the steady state.

(*b*) In the normal animal there is no histological evidence of cell loss from the crypts other than by migration on to the villi, although cellular material may be seen in the lumen of the crypt after treatment with antimitotic agents (Trier, 1962), and after subtotal resection of the small intestine (R. M. Clarke, unpublished observations).

It seems likely, therefore, that in the present investigation of normal rats, the equation

$$\text{Rate of cell production per crypt} = \text{rate of accumulation of colchicine-metaphases per crypt}$$

is valid.

It is not unexpected that the rate of cell production/crypt shows no obvious trend as the small intestine is traversed, since Altmann & Enesco (1967) demonstrated a constant crypt epithelial turnover-time along the intestine. It is interesting to note that the deeper crypts of the duodenum do not have a cell production rate higher

than that of the shallower crypts of the ileum, although, in the case of site 1, the crypt cell population is probably no greater, since the crypts here are of smaller diameter than elsewhere in the small intestine.

The second innovation is the estimation of the crypt/villus ratio. The rate of cell production per villus at any site is the product of the rate of cell production per crypt and the number of crypts per villus, again assuming that, under normal conditions, no cell loss takes place at the crypt-villus junction. There is no histological evidence for this, and the recent work of Loehry, Croft, Singh & Creamer (1969) supports the assumption. The similarity of shape of the plots of villus height and of crypt/villus ratio against distance along the small intestine suggests that there may be a relation between villus height and cell production rate per villus, and these are plotted one against the other in Fig. 9.

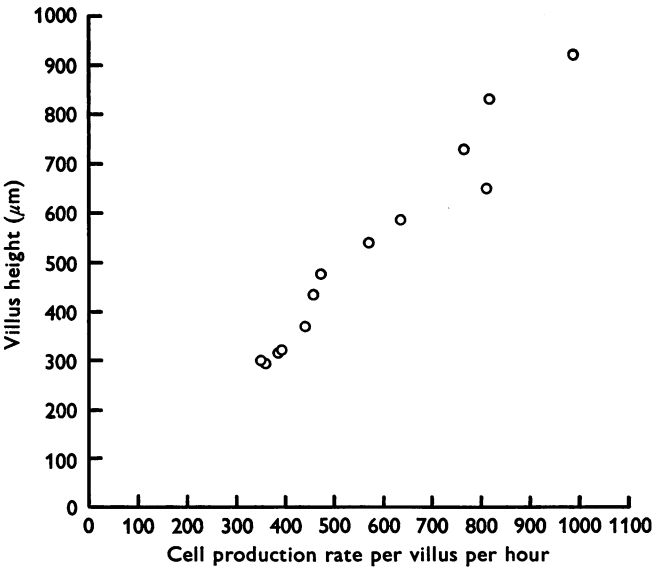


Fig. 9. Abscissa: cell production rate in cells per villus per hour (product of cells per crypt per hour and crypt/villus ratio); ordinate: villus height in μm.

There is a clear correlation between villus height and cell production rate per villus, but this is not specially meaningful, since the height of the villus is not necessarily an accurate reflection of the cell population of the villus. The latter is the basic parameter of interest, since, in the steady state:

$$\text{Cell production rate per villus} = \text{cell loss rate per villus} = \frac{\text{cell population of villus}}{\text{villus transit time}}.$$

It is likely that there are no gross changes in cell packing at different points on the villus (Clarke, 1969), and, if so, then the surface area of the villus may be a guide to the size of the cell population of the villus. The former may be computed from a knowledge of the shape and dimensions of the villus. Examination of a three-dimensional reconstruction of serial longitudinal sections of the terminal ileum (cut

in a plane parallel with the muscle layers) suggested that the villus of the terminal ileum could be considered as resting on a parallel-sided base with semicircular ends, and rising up with an elliptical profile (Fig. 10). Examination with the dissecting microscope of villi from other parts of the small intestine suggested that this model would apply reasonably well as an approximation throughout the intestine. For each

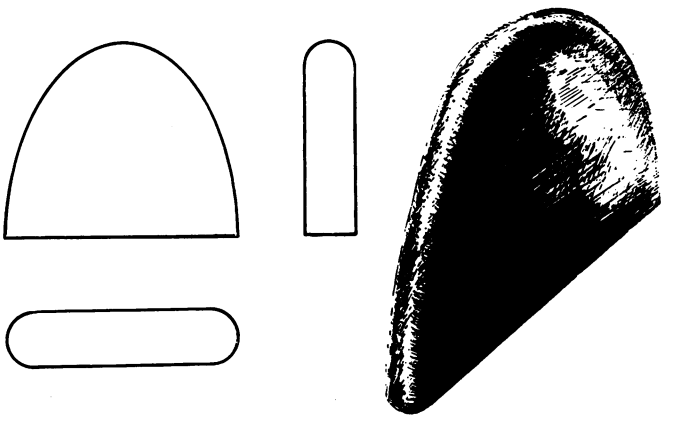


Fig. 10. Diagrammatic representation of shape of villus assumed for calculation of surface area of villus.

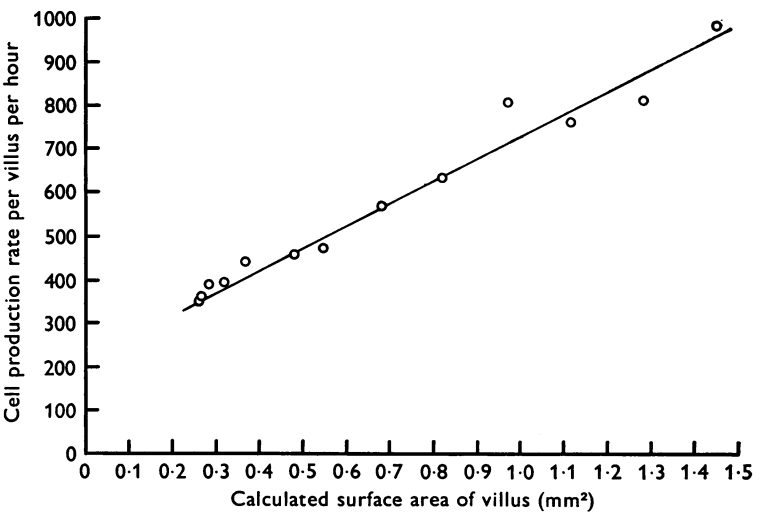


Fig. 11. Abscissa: surface area of villus in mm² (for method of calculation, see text); ordinate: cell production rate per villus per hour (calculated as for Fig. 9). Regression line calculated by method of least squares.

site, from a knowledge of the villus height and thickness and the number of villi per mm², was calculated the surface area of a villus, and in Fig. 11 this is plotted against the cell production rate per villus for that site.

The slope of the regression line calculated by the method of least squares has

a value of approximately 0·5, and since we have measured the cell production rate, and made an estimate of villus surface area, we should be able to calculate the relative transit times for different sites, and compare them with those actually measured by other investigators.

The total villus transit time has not often been measured in rats at more than one site in the small intestine. There appears to be no great difference between the total villus transit time for different stations in the adult rats examined by Koldovsky, Sunshine & Kretschmer (1966), nor in the control rats of Loran & Althausen (1960). The transit time for the terminal ileum in Leblond & Stevens's (1948) investigation is 86 % of that of the duodenum, and the corresponding figure for the terminal ileum in 93-d-old mice is 72 % (Fry, Lesher, Kisieleski & Sacher, 1963), although the mid-intestinal villus transit time did not differ from that of the duodenum. Altmann & Enesco (1967), who also used colchicine-blockade, calculated that the villus epithelial transit time in the duodenum of the adult rat was more than twice that in the terminal ileum, and observed a pattern of decline approximately similar to that shown here. Creamer, Shorter & Bamforth (1961) stated, from the examination of autoradiographs of tissue from mice treated with tritiated thymidine, that the villus transit time in the ileum was 24 h, compared with 48–72 h in the duodenum and jejunum.

Thus there is no unanimity amongst the published ratios of villus epithelial transit time at different stations in the small intestine, and it is difficult to assess which of these investigations presents the true picture. The present work is in good agreement with the experimental results obtained by Creamer *et al.* (1961), and by Altmann & Enesco (1967). From Fig. 11 we can calculate that the transit times for site 1 (proximal duodenum) and site 99 (terminal ileum) should be in the ratio

$$\frac{1{\cdot}44}{985} : \frac{0{\cdot}27}{362} = 1{\cdot}00 : 0{\cdot}50.$$

Thus the results of at least some previous investigations support the conclusions reached by this attempt to shed new light on the nature of the relationship between cell production rate and mucosal architecture in the small intestine of the rat. The biological mechanisms which exist to maintain this relationship are obscure, but quantitative investigations of the rates of cell production and cell loss will be vital in their elucidation.

SUMMARY

1. The rate of cell production in the crypts of Lieberkühn of the small intestine of the adult rat has been assessed by microscopy of individually dissected crypts from fixed tissue, stained in bulk with the Feulgen reaction, after blockade of mitosis with Colcemid.

2. The rate of cell production per crypt is relatively constant along the length of the small intestine, and equals 36 cells/crypt/h.

3. The ratio of crypts to villi has been assessed by microscopy of bulk Feulgen-stained tissue. From 27 crypts per villus in the duodenum, the ratio falls to 10 in the terminal ileum. The product of the crypt/villus ratio and the rate of cell production per crypt is the rate of cell production per villus.

4. An attempt has been made to correlate rates of cell production, estimated surface area of villus, and villus transit times for different parts of the small intestine.

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