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The time-course of changes in mucosal architecture and epithelial cell production and cell shedding in the small intestine of the rat fed after fasting

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INTRODUCTION

The mechanism of control of the replacement of the intestinal epithelium is unknown; it has been suggested that the size of the villus epithelial cell population influences the rate of cell production in the crypts by negative feedback (Galjaard, Meer-Fieggen & Giesen, 1972), but an alternative hypothesis is that the rate of cell shedding from the villus tip contributes to the control of crypt cell proliferation (Clarke, 1974*a*).

Intestinal epithelial cell production is reduced in starvation (Wiebecke, Heybowitz, Löhrs & Eder, 1969; Clarke, 1970; Altmann, 1972; Aldewachi, Wright, Appleton & Watson, 1974) and rises on refeeding (Altmann, 1972; Aldewachi *et al.* 1974). The measurement of cell shedding has not often been undertaken (Loehry, Croft, Singh & Creamer, 1969; Goldsmith, 1969; Clarke, 1970), but the rate of cell shedding in starved rats is consistent with the cell production rate (Clarke, 1970), and is therefore less than the rate of cell shedding in fed animals.

If cell shedding does contribute to the control of cell production, then refeeding a starved animal should cause an increase in cell shedding, which should precede an increase in the rate of cell production. This paper describes an experiment to investigate the time-course of changes in cell shedding and cell production when starved rats were allowed access to food. Such measurements have not previously been made simultaneously in the same animal, and apart from the study of Aldewachi *et al.* (1974), little attention has been paid to the events occurring soon after refeeding is permitted.

MATERIALS AND METHODS

Conventionally reared male albino Wistar rats were obtained from the Nottingham University Joint Animal Breeding Unit, Sutton Bonington, Leics., at least 2 weeks before use, and fed on Diet 41B (Pilsbury, Birmingham) and tap water *ad libitum*. They were then housed individually in cages with wire mesh floors and for 72 hours had access to tap water alone. They were then allowed to feed (09.00 hours) on pellets of a low-residue diet, since preliminary experiments showed that it was not possible to wash Diet 41B out of the intestine *in vivo* without the use of excessive pressure. The diet contained sucrose 79 %, casein 15 %, cooking oil (Rakusen) 3 %, sodium chloride 1 %, gum tragacanth 1 %, gum acacia 1 % (w/w). Control animals continued to fast.

Animals were injected intraperitoneally with a solution of Colcemid (Ciba), 2·5 mg/kg body weight, and anaesthetized by intraperitoneal injection of pentobarbitone sodium (B. Vet. C.) between 15 and 135 minutes later. Cell shedding was estimated in the manner described by Clarke (1970), except that the saline solution was 'chased out' by fixative after 5 minutes in every animal. The interval between Colcemid injection and fixation was noted. Villus height, crypt depth, crypt/villus ratio and cell production rate per crypt were measured, as described by Clarke (1974*b*), at a single site 10% of the way down the small intestine from the pylorus, near the ligament of Treitz. All specimens were examined without knowledge of the treatment received by the animal. Groups of at least six experimental (refed) and control (fasted) rats were killed ½, 1, 2, 4, 6, 9, 12, 16 and 24 hours after refeeding. In addition, the intestine was washed out retrogradely, i.e. from ileocaecal junction to pylorus, in a group of six rats 4 hours after refeeding.

Specimens of intestine from similar sites were taken from the animals (refed and control) killed 4 hours after refeeding; after fixation, dehydration and clearing they were embedded in paraffin wax and sectioned at 5 μm, as described by Clarke (1975). Briefly, this involved sectioning a cylinder of gut so that villi were cut successively in transverse and in longitudinal section, so that counts could be made of the number of epithelial nuclei along the base of the villus and from base to tip respectively. Counts in each direction were made on ten villi in six animals from each (refed and control) group. In each set of sections where the villi were cut longitudinally, the number of nuclei lining the crypts of Lieberkühn from base to crypt-villus junction were also counted ($n = 10$).

Eight rats were fed pellets of a similar diet to which powdered charcoal had been added (5%, w/w). They were killed at intervals and examined to see how far down the intestine the charcoal had progressed.

RESULTS

Intestinal transit. Charcoal (and presumably the other constituents of the diet) passed rapidly down the proximal small intestine, reaching the half-way point 10 minutes after feeding, but slowing distally; this confirms the findings of Wiepkema, Alingh Prins & Steffens (1972) and Poulakos & Kent (1973).

Villus height (Fig. 1). Two hours after refeeding, the villi of refed rats were taller than those in starved controls, and had reached the height of villi in fed rats by 4 hours. The height fluctuated thereafter, but was significantly greater than in starved controls ($P < 0{\cdot}05$; Student's t test) at 2, 4, 6, 9, 12 and 24 hours after refeeding. The villi did not appear to be different in shape in the two groups of animals.

Crypt depth (Fig. 1). Crypt depth was below fed values in both refed and control animals, and was still below 24 hours after refeeding. Altmann (1972) made similar observations 24 hours after refeeding, but Aldewachi *et al.* (1974) reported a prompt increase in the size of the crypt cell column after refeeding.

Crypt/villus ratio (number of crypts per villus). This did not alter significantly during the experiment in refed or control animals.

Cell production rate (Fig. 1). This fluctuated between 9 and 16 cells/crypt/hour in both groups until 12 hours after refeeding, when the rate in refed animals rose to

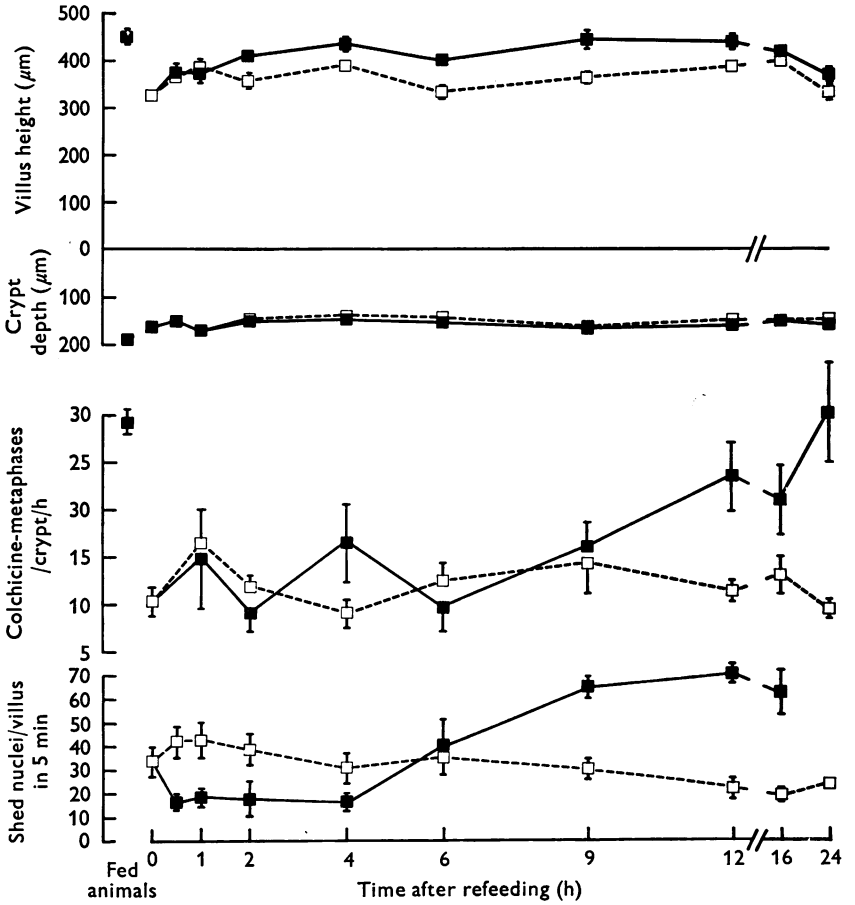

Fig. 1. The time-course of changes in the proximal small intestine after refeeding. Abscissa: time after refeeding (hours); ordinate (from above downwards): villus height (μm), crypt depth (μm), colchicine-metaphases/crypt/hour and number of nuclei trapped/villus in 5 minutes. Each point represents the mean of a group of at least six animals; □, fasted; ■, fed or refed (initial solid square indicates control values in fed rats). Bar represents ±1 S.E.M., except for colchicine-metaphases/crypt/hour, where it represents the standard error of the slope of the regression of colchicine-metaphases/crypt against time (Snedecor & Cochran, 1967).

23·3 ± 3·7 cells/crypt/hour, significantly greater than the rate in control animals (11·3 ± 1·1; $P = 0{\cdot}02$, F-test; Snedecor & Cochran, 1967). The rate of cell production in refed animals had reached the fed level by 24 hours after refeeding.

To examine whether there was any spurt of cell production very soon after refeeding (perhaps due to G2 cells in the crypts) the animals killed ½ hour after refeeding were injected with Colcemid at the onset of refeeding. There was no significant difference (Student's t test) between the counts of colchicine-metaphases per crypt in refed and control animals.

Cell shedding (Fig. 1). The rate of cell shedding was assessed by counting the number of nuclei shed from the epithelium and trapped in the mucus at the top of a

villus after 5 minutes' exposure to the saline solution in the intestinal lumen. This does not allow the calculation of an absolute rate, but it does allow the comparison of events in refed and control animals.

The number of nuclei shed per villus was reduced within half an hour of refeeding ($P < 0{\cdot}05$ for $\frac{1}{2}$ and 1 hour values; Student's t test), and remained at a low level until 3 hours after refeeding. At 4 hours the number of nuclei shed per villus was not different in the groups with intestines washed out in the normal or in the retrograde direction. From 9 hours, the number of shed cells per villus was significantly greater in refed animals ($P < 0{\cdot}01$). Even with the use of the low-residue diet, it proved impossible to wash out the gut in animals 24 hours after refeeding.

No shed cells were seen during microscopic examination of samples of the fluid used to wash out the intestine.

Measurements on sectioned villi from 4 hour animals. The number of nuclei counted from crypt–villus junction to villus tip was $72{\cdot}3 \pm 3{\cdot}1$ (mean ± S.E.M.) for animals 4 hours after refeeding, compared with $63{\cdot}7 \pm 1{\cdot}3$ for the control animals ($P < 0{\cdot}05$). The number of nuclei from crypt–villus junction to crypt base was unchanged ($21{\cdot}6 \pm 0{\cdot}5$ compared with $21{\cdot}7 \pm 0{\cdot}3$). The number of nuclei counted along one side of a villus transversely sectioned through its base was $78{\cdot}4 \pm 3{\cdot}3$ in the refed, and $72{\cdot}9 \pm 3{\cdot}0$ in the controls ($P > 0{\cdot}10$).

There was thus a significant increase in villus cell column height 4 hours after refeeding, with a much smaller increase in the number of cells along the base of the villus, and no change in the crypt cell column height. Since the number of nuclei in both the height and the width of the villus increased, it is likely that the villus epithelial population size increased also, since the shape of the villi appeared unaltered.

DISCUSSION

Goldsmith (1973) measured the rate of DNA accumulation in the intestinal lumen of fasted and five refed cats, but the DNA washout technique can be criticized as a method of measuring cell shedding rate (Clarke, 1970).

Willems, Vansteenkiste & Smets (1971) observed a reduction in foveolar height in the dog gastric mucosa within 4 hours after refeeding, which they ascribed to post-prandial cell shedding.

In the present study, during the first 4 hours after refeeding, the intestine was presumably motile, and active in secretion, digestion and absorption; and an increase in cell shedding, whether from abrasion or other causes, might have been predicted. Thus, the unexpected finding in this investigation was the *reduced* rate of cell shedding during the first 4 hours after refeeding, as shown by the consistently smaller number of nuclei trapped in the mucus on the villi after 5 minutes' exposure to the saline solution. The reduction in the number of nuclei trapped in mucus on villi in the proximal intestine might possibly be due to their having been dislodged from the mucus; if this were so, since they did not appear in the washout fluid, they must have been trapped on villi further down the intestine. Examination of specimens taken from the distal parts of the intestine did not confirm this suspicion. In addition, any dislodged cells might be expected to appear in the washout fluid when the intestine was washed out in a retrograde direction. They did not do so. Thus, the

most likely explanation for the reduction in the number of trapped nuclei is that there was a genuine reduction in the rate of cell shedding.

Therefore it may be taken that cell shedding did not increase until after 4 hours, and was not significantly different from that in starved controls until 9 hours after refeeding.

Since cell production does not start to increase during the first 9 hours, both the absolute size of the villus, and the size of its epithelial cell population, should be greater in the refed gut than in the control. Both of these measurements were greater in refed than in starved control animals 4 hours after refeeding. Since the extra cells are unlikely to have come from the crypts, which did not change in length, these findings support the reality of an initial reduction in cell shedding after refeeding.

Cell production does not appear to exceed control values until more than 9 hours after refeeding. Aldewachi *et al.* (1974) found a steady increase in mitotic index in the crypts of refed rats, and they found a higher cell production rate at 16 hours (also measured stathmokinetically) than in this investigation. However, they fed their rats on their accustomed diet, and they also refed at different times of day, and fixed the gut at a constant time, whereas the experiment described here used a constant feeding time and different fixing times.

In starved, newly hatched chicks, Cameron & Cleffmann (1964) observed a delay of 8 hours after refeeding before mitotic activity in the duodenum began to increase. Willems *et al.* (1971, 1972) demonstrated a slow rise in mitotic activity in gastric biopsies 16 hours after refeeding dogs, both in the intact mucosa (1972) and in a Heidenhain pouch (1971); they ascribed the latter result to the action of circulating gastrin. It would be interesting to compare the time-course of cell production in a surgically isolated sac of small intestine with that in intestine-in-continuity in a starved rat allowed to refeed.

There was no evidence in this study for a population of crypt cells stalled in G2; the evidence of Pedersen & Gelfant (1970), proposing the existence of such a population, is unsatisfactory because unlabelled mitoses are not unexpected in the 4 μm sections which they used for ^{3}H autoradiography. Therefore it is likely that the increase in cell production arises either by acceleration of cells through the cycle or by mobilization of cells in G1. Aldewachi *et al.* (1974) showed an increased rate of entry of cells into mitosis 16 hours after refeeding, which is consistent with a reduction in cell cycle time, but the absence of a clear increase in cell production rate in this study in the first 9 hours suggests the mobilization of a cohort of cells, rather than a general acceleration of cells throughout the entire cycle, which would result in a gradual build-up in cell production from an early stage. This could be due to gradual mobilization of a G0 cohort (cells out of cycle) or of G1 cells by acceleration of the entry into S. If such a cohort of either type of cells is mobilized, we would not expect any extra cells to reach mitosis until they had passed through S and G2 and at least part of G1. S and G2 together total 7·5 hours in fed rats (Cairnie, Lamerton & Steel, 1965), and the cell cycle is prolonged in starvation (Wiebecke *et al.* 1969; Aldewachi *et al.* 1974); it is therefore improbable that the mobilization of these cells for division begins more than 1·5 hours (i.e. 9–7·5 hours) after refeeding. Cameron & Cleffmann (1964) and Aldewachi *et al.* (1974) showed increases in labelling index

2 hours after refeeding. Since the increase in cell shedding does not occur until more than 4 hours after refeeding, it does not seem likely that the increased rate of cell shedding could be a stimulus to increased cell production. It would be instructive to measure the *rate* of entry of cells into S during the early period after refeeding.

Nor is Galjaard's (Galjaard *et al.* 1972) hypothesis that the size of the villus epithelial population controls cell proliferation supported by these findings, since, according to this hypothesis, the increase in villus cell column height that occurs 4 hours after refeeding should reduce cell proliferation, and the villi are also persistently taller in refed animals than in controls from 2 hours onwards, that is during the period of the presumed cellular preparation for the increase in cell production which is first seen at 12 hours.

This experiment therefore gives support neither to the hypothesis that increased cell shedding from the villus tip is a possible contributory stimulus to increased cell proliferation in the crypts, nor to the hypothesis that villus epithelial population size is an important regulator of crypt cell proliferation rate under these experimental conditions.

SUMMARY

Rats were starved for 3 days, then either allowed access to food or continued in starvation. The following measurements on the upper jejunum were made on groups of refed and starved rats at nine time intervals after refeeding: villus height, crypt depth, crypt/villus ratio, rate of cell production per crypt, and number of epithelial cells shed per villus in 5 minutes.

Villus height increased 2 hours after refeeding, while crypt depth changed less dramatically. Crypt/villus ratio was unchanged.

The number of shed epithelial cells per villus was reduced below the starved level for 4 hours after refeeding, and did not rise significantly above the starved level until 9 hours; the rate of cell production was not significantly increased until 12 hours after refeeding. No evidence for a reserve of cells in G2 was found, and the 3 hour lag between the rise in cell shedding and the increase in cell production would probably not give enough time for cells in G1 to pass through S and G2. It is concluded that these observations do not support the hypothesis that increased cell shedding from the top of the villus stimulates increased cell production in the crypts of Lieberkuhn.

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