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The effect of starvation and refeeding on cell population kinetics in the rat small bowel mucosa

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INTRODUCTION

In recent years there has been increasing interest in the control of cell proliferation, especially in renewal systems such as the small intestinal mucosa. While studies of cell kinetics under normal physiological conditions have proved valuable (Cairnie, Lamerton & Steele, 1965*a*, *b*), more information about the control mechanisms has come from experiments designed to provoke the renewal systems into making a series of compensatory adjustments. One such stimulus which has received a good deal of attention is starvation.

The morphological sequelae of starvation in the rat have been described by Brown, Levine & Lipkin (1963) and comprise villous tip destruction, with basal fissuring and shortening of villi. A decrease in the villous cell population was also suspected by Stevens Hooper & Blair (1958) whose studies further demonstrated a reduction in crypt cell population in rats starved for 5 days. Altmann (1972) confirmed the reduction in crypt and villous size after 5 days' starvation, while Hopper, Wannamacher & McGovern (1968) reported a 33% decrease in crypt population in the rat after 10 days' starvation. On a broader front, Clarke (1972) found a small reduction in the total number of crypts in rats starved for 5 days.

Accompanying this hypoplastic response there were considerable changes in crypt cell kinetics: Weibecke, Heybowitz, Lohrs & Eder (1969) showed a reduction in labelling and mitotic indices in mice starved for 4 days, due to an increase in the duration of the cell cycle; Rose, Hopper & Wannamacher (1971) demonstrated only a small increase in the duration of DNA synthesis in starved rats, but Altmann (1972) reported a prolongation of turnover time, in similar circumstances.

These starvation-induced hypoproliferative responses in the small bowel mucosa have been wholly attributed to the increase in cell cycle time (Hopper, Rose & Wannamacher, 1972), but most workers have overlooked the important role of the *growth fraction*, i.e. the proportion of crypt cells engaged in proliferation. Changes in this parameter, as well as in the cell cycle time, control the rate of crypt cell production, and a reduction in the growth fraction could contribute to the demonstrated reduction in proliferative indices. If this is the case then some proliferating crypt cells may subside into a resting state during starvation and return to activity on the resumption of feeding.

A third possibility arises from the studies of Cameron & Cleffman (1964): when starved chicks were fed the labelling index rose almost immediately, but the mitotic index rose only after an interval of 8 hours; this indicated that cells were arrested in the G_1 phase of the cell cycle, and had to complete DNA synthesis before entering mitosis.

It therefore becomes necessary to differentiate between the relative contributions of the established increase in cell cycle time, and a possible decrease in proliferating population, to the hypoproliferative response to starvation; and also to determine whether or not there is a specific control point in G_1 at which cells become blocked in their progress around the cell cycle. In this paper we consider the effects of starvation and refeeding on proliferative indices, growth fraction and cell cycle time in the rat small bowel.

MATERIALS AND METHODS

Male albino Wistar rats aged 3 months and weighing 150–200 g were used throughout. In the labelling experiments animals received 0·5 μCi per g body weight of tritiated thymidine, ^{3}HTdr (Radiochemical Centre, Amersham, England) by intraperitoneal injection 1 hour before death. The ^{3}HTdr was of specific activity 5 Ci/mM, and the concentration used was 100 μCi per ml. In the stathmokinetic experiments each animal was given 1 mg per kg body weight of vincristine sulphate (Eli Lilly and Co. Ltd.) by intraperitoneal injection. The rats were killed by cervical dislocation. Samples of small bowel were taken from the region immediately below the ligament of Treitz, and may thus be described as upper jejunum. The tissue was fixed in Carnoy's fluid for 6 hours and processed through to paraffin. Transverse sections were cut at a thickness of 3 μm and stained with Harris' haematoxylin.

Autoradiographs were prepared using Kodak AR 10 stripping film and exposed for 28 days; the slides were developed using Kodak D19b and were then fixed, washed and stained through the emulsion with Harris' haematoxylin.

Experiment 1

Rats were starved continuously for 96 hours starting at 0900 hours, but were allowed free access to water. Animals received ^{3}HTdr one hour before death, and one animal was killed every four hours during the experimental period.

Experiment 2

After 96 hours starvation, again starting at 0900 hours, rats were allowed free access to food. After this comparatively short period of starvation the animals responded by immediate resumption of eating. During the first 24 hours after refeeding one animal was killed every two hours, and subsequently every three hours up to 48 hours after refeeding. Animals again received ^{3}HTdr one hour before death.

Experiment 3

Stathmokinetic studies were carried out at 96 hours after starvation, and after 16 hours refeeding of rats previously starved for 96 hours. In an attempt to minimize

the effects of diurnal variation (Sigdestad, Bauman & Lesher, 1969) vincristine was consistently injected at 0900 hours. Animals were killed at intervals of 15 minutes up to 150 minutes after vincristine. In each animal the mitotic index was measured by studying 100 crypt sections as detailed below.

Counting techniques

In each animal the labelling index and/or the mitotic index was obtained by analysing 100 axially sectioned crypts. In each crypt the left hand column of cells was numbered, counting from the bottom upwards to the crypt–villus junction, and the positions in this *crypt column* of labelled or mitotic nuclei were recorded. A cell was regarded as labelled if 5 or more grains were localised over the nucleus, and the criteria for the recognition of the various mitotic stages were those of Clarke (1970). In the animals killed 15 minutes after vincristine injection a few telophases were still apparent; but thereafter only prophases and metaphases were seen and counted.

The data given by these techniques allowed construction of *labelling and mitotic index distribution curves* (Cairnie *et al.* 1965*a*) in which the labelling or mitotic index was plotted as a function of cell position in the crypt. The variation in the height of the crypt columns (Cairnie & Bentley, 1967) was compensated for by analysing the data by an ALGOL computer program run on an IBM 360/67 computer. The details of the method are given elsewhere (Wright, Morley & Appleton, 1972*a*, *b*).

In the crypts two morphometric parameters were measured: the length of the crypt column in terms of cell number was measured in the analysis of the axially sectioned crypts described above; the number of cells in cross sections of crypts is the *column count* (Cairnie, 1967) which was measured by counting 100 crypt cross sections in each animal. The product of the crypt column length and the column count gives an estimate of the *total crypt population* (Wimber & Lamerton, 1963; Cairnie, 1967).

RESULTS AND INTERPRETATION

Morphometric parameters

After starvation the crypt column length had decreased from $33{\cdot}1 \pm 0{\cdot}3$ (S.E.) cells at 4 hours to $29{\cdot}9 \pm 0{\cdot}2$ cells at 96 hours. Fig. 1 shows that the crypt column length decreased slowly over the experimental period. This was accompanied by a small reduction in the column count (Fig. 1) which fell from $20{\cdot}1 \pm 0{\cdot}1$ cells to $19{\cdot}3 \pm 0{\cdot}1$ cells. The total crypt population, calculated from the product of the crypt column length and the column count, showed a reduction from 670 cells to 580 cells after 96 hours' starvation, a 12% reduction (Fig. 1).

With the resumption of feeding, the column count showed a slow return towards control values by 48 hours after refeeding (Fig. 2). The crypt column length, after an initial increase during the first 12 hours of refeeding, fell again over the remainder of the experimental period. The net effect of these changes on the total crypt population is shown in Figure 2: after some variation over the first 24 hours of refeeding, the crypt population was maintained at a constant value slightly below the control value.

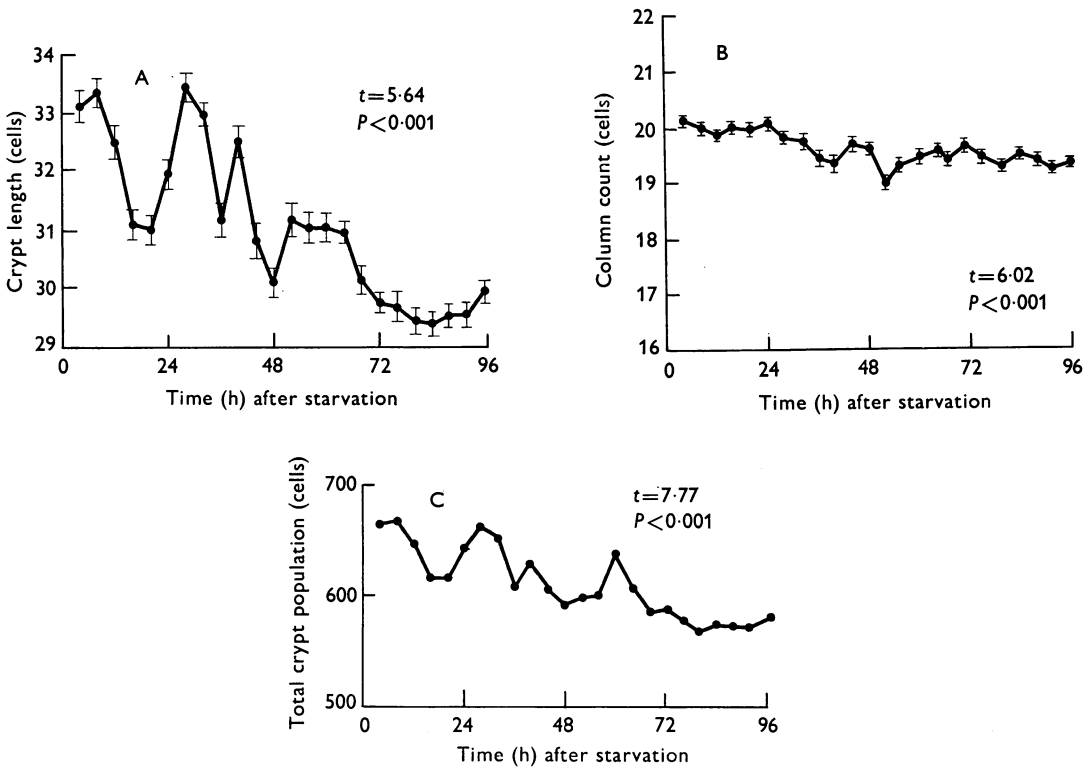

Fig. 1. Changes in crypt column length (± S.E.), column count (± S.E.) and total crypt population during 96 hours' starvation in male rats.

Proliferative indices

The labelling index (Fig. 3) showed a steady fall over the 96 hours of starvation, decreasing from 36·4% to 23·3%. The mitotic index appeared to remain constant over the first 40 hours and then decreased from values above 6·0% to reach 4·5% after 96 hours' starvation.

Upon refeeding the labelling index increased quickly to reach a peak of 34·5% after 12 hours (Fig. 4); this was followed by a small decrease over the next 10 hours before constant values of around 30% were realized for the remainder of the experiment. In the case of the mitotic index a latent period of approximately 6 hours was apparent before the mitotic index began to rise steadily to a peak of 9·6% at 16 hours.

The fall in proliferative indices during the period of starvation was gradual and whatever mechanism was responsible for the fall operated slowly. On the other hand, compensatory changes after refeeding were rapid. While the present data did not admit of definitive interpretation, we may speculate that the first kinetic event in the proliferative response was an increased flow of cells into DNA synthesis, and later into mitosis. It is evident that this postulated sequence could be brought about by the resumption of the progress into S of cells which were originally arrested in G

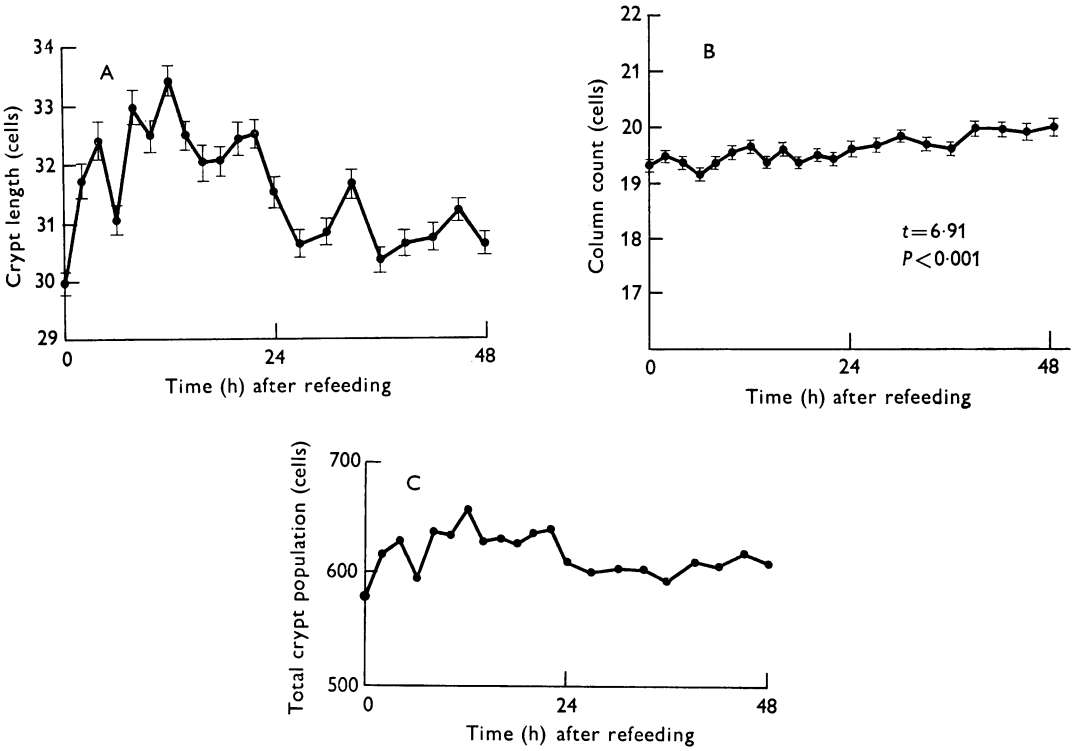

Fig. 2. Changes in crypt column length (±S.E.), column count (±S.E.) and total crypt population with time after refeeding male rats starved for 96 hours.

or in a G_0 compartment, or by an increased G_1 to S flow due to a reduction in the duration of G_1.

Proliferating populations

Fig. 5*a* shows the labelling index distribution curve for the animal killed 4 hours after starvation. Low values were found in the bottom few cell positions, followed by a peak of over 75% in cell positions 5–9. Thereafter a steady decrease took place over the remaining cell positions. By drawing a perpendicular at the cell position of the 50% peak value, an estimate could be obtained for the crypt growth fraction (the fraction of crypt cells actually engaged in the cell cycle). A growth fraction (I_p) calculated for the whole crypt represented the fraction of the crypt population which occupied the proliferative compartment; this was the region over which labelled cells were observed, and may be considered to end at approximately the cell position where the labelling index fell to 50% of its maximum value (Cleaver, 1967). Wright *et al.* (1972*a*) showed good agreement between values calculated by this method and those obtained from FLM curves. In Fig. 5*a* the 50% peak value is at position 17, and the growth fraction for the whole crypt may be taken as the ratio of the proliferative compartment size of 17 cells to the total number of cells in the crypt, i.e. 17/33 (0·51). Labelling index curves were constructed for each

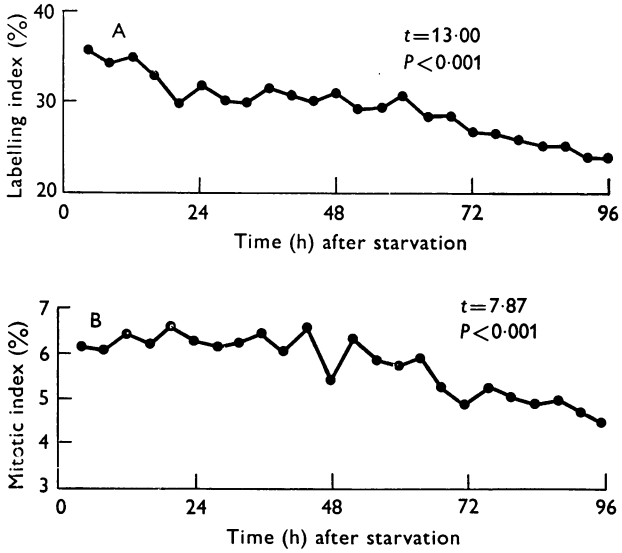

Fig. 3. Changes in labelling and mitotic indices during 96 hours' starvation in male rats.

animal and values of the growth fraction obtained in the same way. The growth fraction as a function of time after starvation is shown in Fig. 6, with the labelling index distribution curve for the animal killed at 96 hours depicted in Fig. 5*b*.

The growth fraction at 96 hours was 0·52, almost the same as that at 4 hours, and it can be seen from Fig. 6 that throughout the duration of the starvation period I_p remained between 0·50 and 0·55. It was concluded that the changes in growth fraction did not contribute to the demonstrated reduction in proliferative indices.

Changes in the growth fraction with time after refeeding are shown in Fig. 7. After a small initial decrease only a slight rise was apparent, with a peak value of 0·55 at 8 hours after refeeding. This was followed by a fall to reach a substantially constant value of around 0·5 during the remainder of the experiment. This increase in I_p during the initial phases of the proliferative response was not significant, and it could be proposed with confidence that such a small increase in the growth fraction could not play an important part in the rise in proliferative indices after refeeding. Labelling index distribution curves are shown for the animals killed at 8 and 16 hours after refeeeding in Figs. 5*c* and 5*d*.

An estimate of the total number of proliferating cells per crypt could be obtained from the product of the growth fraction and the total number of cells in the crypt, and the changes during starvation are shown in Fig. 8. A small decrease from about 350 cells per crypt to 300 occurred over the 96 hours; as the growth fraction remained substantially constant during this period, this reduction in total proliferating cells was a direct reflection of the decrease in crypt population (Fig. 1). Upon refeeding (Fig. 9) an increase to control values occurred initially, due largely to the small increase in growth fraction (Fig. 7) but also partly to an increase in total crypt population. Values then settled at around 300 cells for the remainder of the experiment.

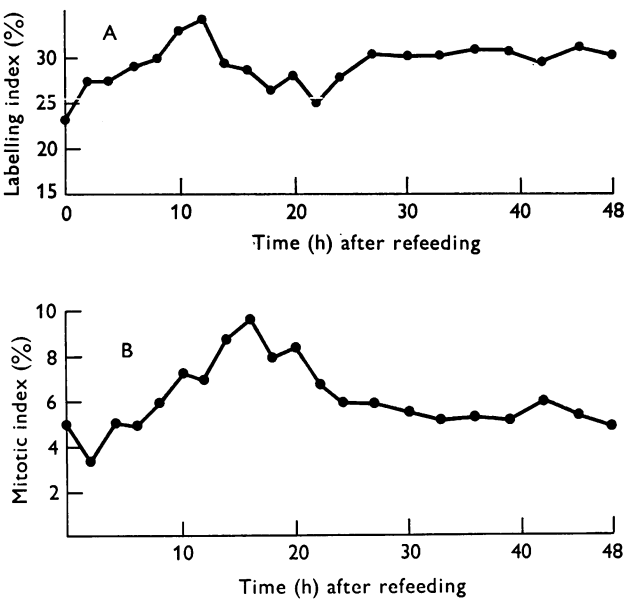

Fig. 4. Changes in labelling and mitotic indices with time after refeeding male rats starved for 96 hours.

Cell cycle parameters

We have shown that considerable decreases in the proliferative indices in the small bowel mucosa can be produced by 96 hours' starvation, followed by rapid rises after refeeding. A hypoplastic response was also evident in the form of decreased numbers of crypt cells. During starvation there was no demonstrable change in growth fraction, and it was therefore necessary to look closely at changes in the cell cycle time.

The mitotic index accumulation line after vincristine injection at 96 hours of starvation is shown in Fig. 10*a*. The readings prior to 30 minutes after vincristine were ignored because of uncertainties concerning the stathmokinetic events during the early stages of the experiment (Clarke, 1971; Pelc, 1971; Wright *et al.* 1972*b*). A good degree of linearity was apparent over the stathmokinetic period.

The slope of the line is a function R of the rate of entry into mitosis and is equal to 5·74 % of cells per hour. For absolute values a further factor should be considered, viz. Tannock's constant (Tannock, 1967; Wright *et al.* 1972*b*) by which R must be multiplied in order to compensate for the spatial arrangement of cells in the crypt. This value in male Wistar rats was 0·62 (Wright *et al.* 1972*b*). Therefore r, the rate of entry of cells into mitosis per proliferating cell of the population, is related to the rate of entry as a percentage by

$$r = \frac{5{\cdot}74}{0{\cdot}52 \times 100} \times 0{\cdot}62 = 0{\cdot}0684. \quad (1)$$

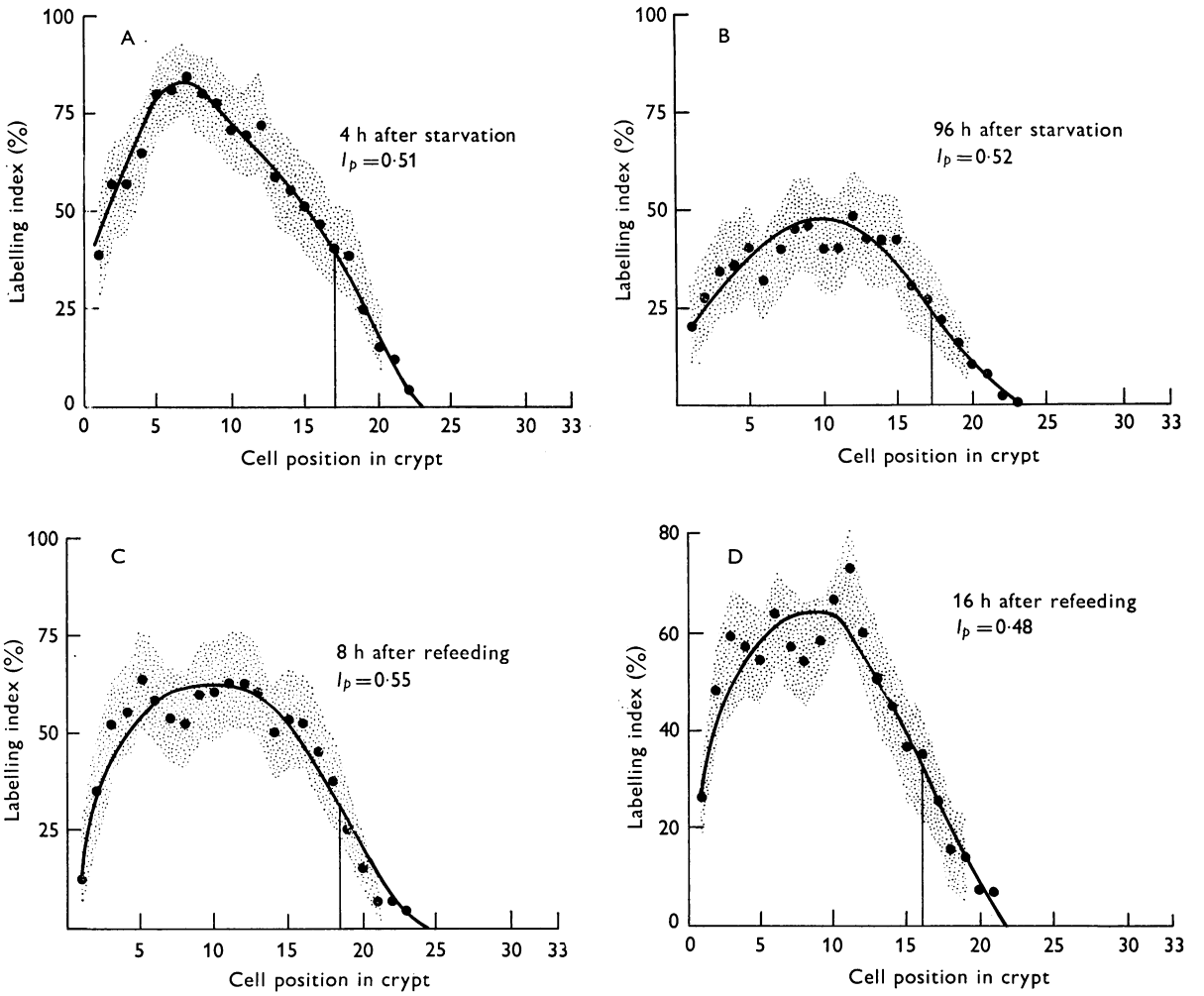

Fig. 5. Labelling index distribution curves (A after 4 hours' starvation, B after 96 hours' starvation, C 8 hours after refeeding, and D 16 hours after refeeding). The shaded areas indicate 95 % confidence limits for the points.

Assuming steady state conditions within the population, the mean cell cycle time (T_c) is the reciprocal of the rate of entry into mitosis or

$$T_c = \frac{1}{r} = \frac{1}{0{\cdot}068} = 14{\cdot}7 \text{ hours}$$

compared with a control value of 10·4 hours (Wright *et al.* 1972*b*); the difference is significant ($P < 0{\cdot}001$), and we concluded that the decrease in proliferative indices evoked by starvation was due entirely to prolongation of the cell cycle time. The mitotic duration (Wright *et al.* 1972*b*) was also prolonged at 1·0 hour, compared with a control value of 0·4 hour.

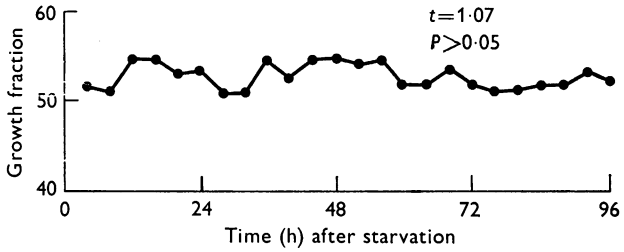

Fig. 6. The growth fraction as measured from the labelling index distribution curve for animals killed during 96 hours' starvation.

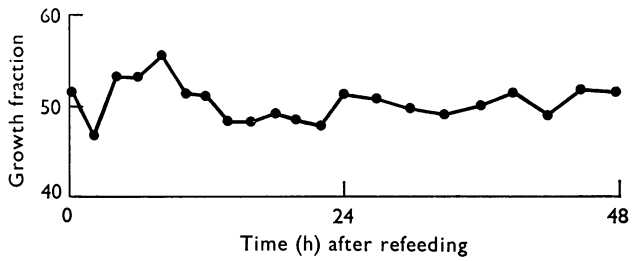

Fig. 7. Growth fraction changes with time after refeeding male rats starved for 96 hours.

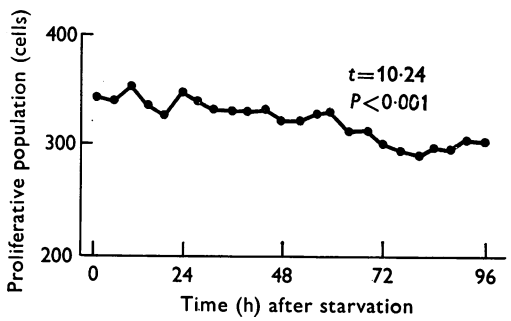

Fig. 8. Total proliferating cells per crypt plotted against time after starvation.

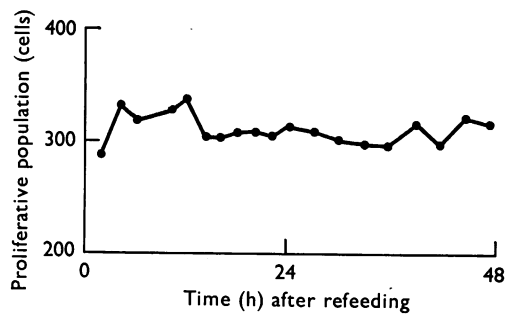

Fig. 9. Changes in total proliferating cells per crypt with time after refeeding male rats starved for 96 hours.

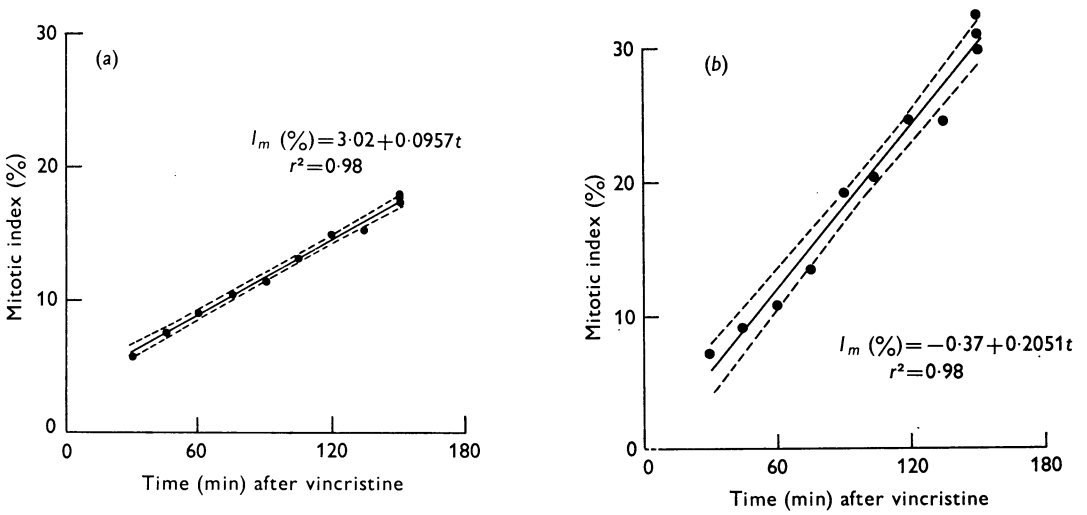

Fig. 10. (*a*) the mitotic accumulation line with time after vincristine injection for the whole crypt column of male rats starved for 96 hours and (*b*) for the whole crypt column of rats after 16 hours' refeeding. The broken lines indicate 95 % confidence limits for the lines.

The mitotic index accumulation graph of 16 hours after refeeding, i.e. at the peak of the mitotic index response, is shown in Fig. 10*b*. Again there is a good degree of linearity. However,the 95 % confidence limits obtained in this experiment were wider than those after 96 hours' starvation (Fig. 10*a*) and in control animals (Wright *et al.* 1972*b*); this could be due to failure to take into account the rate of entry into mitosis in a situation where the mitotic index was itself increasing; in other words, the situation was dynamic with respect to changes in T_c. We return to this point in the discussion. The slope of the line in Fig. 10*b* is significantly greater than that shown in Fig. 10*a* ($P < 0{\cdot}001$).

The cell cycle time, calculated as detailed above, and using the growth fraction measured at 16 hours (0·48), was 6·55 hours. The mitotic duration was also shortened to 0·6 hour. The crude rate of entry into mitosis (R) was 12·3 % of cells per hour. The increase in proliferative indices which occurred after refeeding starved animals was therefore attributable to a reduction in cell cycle time.

The cell cycle parameters measured are summarized in Table 1, together with other parameters measured at the same time intervals.

Flux parameters

Wright *et al.* (1972*b*) have shown that in order to calculate an accurate *cumulative birth rate curve* the mitotic duration must be measured at each cell position. Mitotic accumulation graphs were plotted for each individual cell position in the same way as for the whole crypt column, and the mitotic duration calculated as before (Wright *et al.* 1972*b*).

Table 1. *Changes in cell kinetics in the crypts of Lieberkühn of the rat after* 96 *hours' starvation, and* 16 *hours after subsequent refeeding*

	Crypt population (cells)	Growth fraction	Total proliferating cells per crypt	Labelling index (%)	Mitotic index (%)
Control	670	0·51	340	36·0	6·2
96 h starvation	580	0·52	301	23·3	4·5
16 h refeeding	630	0·48	310	28·5	9·7

	Crude rate of entry into mitosis (%/h)	Cell cycle time (h)	Mitotic duration (h)	Migration rate (cell positions/h)	Cell production rate (cells/crypt/h)
Control	11·2	10·4	0·40	1·8	39
96 h starvation	5·7	14·7	1·0	1·0	19
16 h refeeding	12·3	6·5	0·60	1·9	38

	Transit time (proliferative compartment) (h)	Transit time (maturation compartment) (h)	Transit time (proliferative + maturation compartments) (h)
Control	26	8	34
96 h starvation	83	15	98
16 h refeeding	33	9	42

Cell cycle and flux parameters for control animals are taken from Wright, Aldewachi, Appleton & Watson, in preparation.

The *birth rate* (k_b) at each cell position was then calculated, assuming steady state conditions, from the equation

$$k_b = \frac{I_m}{t_m}. \tag{2}$$

The values for each cell position were added, and the resulting cumulative birth rate was plotted as a function of cell position in the crypt column. Cumulative birth rate curves are shown for rats after 96 hours' starvation (Fig. 11*a*) and after 16 hours' refeeding (Fig. 11*b*). A higher limiting value was apparent in the refed animals.

The birth rate is equal to the rate of cell production (cells/crypt/hour) at each cell position; consequently the maximum value attained is the rate of cell production for the whole crypt column. An estimate of the *crypt cell production rate* may be obtained from the product of the cell production rate per crypt column and the number of columns in the crypt, the column count.

After 96 hours' starvation the crypt cell production rate was reduced to 19 cells/crypt/hour, compared with a value for control, normally fed rats of 39 cells/crypt/hour (see Table 1). Sixteen hours after refeeding the starved animals, the crypt cell production rate was a near normal at 38 cells/crypt/hour. Assuming that movement of cells within the crypt resulted only from crypt cell proliferation

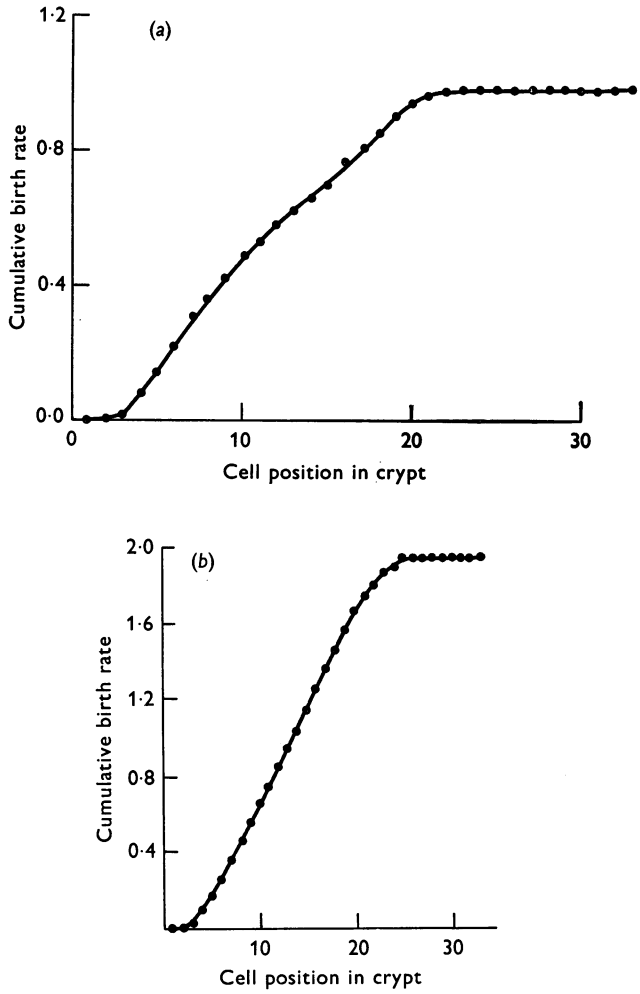

Fig. 11. Cumulative birth rate curves for (*a*) rats after 96 hours' starvation, and (*b*) rats after 16 hours' refeeding.

(Cairnie, Lamerton & Steele, 1965*b*) then the maximum birth rate attained was also equal to the migration rate at the top of the proliferative compartment. This was 1·0 cell positions/hour after 96 hours' starvation, increasing to 1·9 cell positions/hour 16 hours after refeeding. The value for control, normally fed rats was 1·8 cell positions/hour.

The size of the proliferative and maturation compartments in terms of the crypt column was measured from the labelling index distribution curves. The growth fraction, and hence the relative size of the proliferative compartment, remained substantially constant throughout both starvation and refeeding, but there was an absolute decrease in proliferative compartment population because of the reduction

in crypt size. The *transit times* through these proliferative and maturation compartments could be calculated from the cumulative birth rate curves, the reciprocal of the cumulative birth rate at each position being equal to the time taken to traverse that position. Adding these reciprocals over the relevant portion of the crypt gave transit times. In the rats starved for 96 hours, the transit time through the proliferative compartment was increased to 83 hours compared with our control values of 26 hours, and maturation compartment transit time was also increased from a control value of 8 hours to a value of 15 hours after 96 hours' starvation. In the refed animals on the other hand, transit times were reduced to 33 hours in the proliferative compartment, and 9 hours in the maturation compartment. These values are summarized in Table 1.

DISCUSSION

This study has shown that during starvation in the rat there is a reduction in crypt population. This hypoplastic response confirms the earlier findings of Stevens Hooper & Blair (1958), Hopper *et al.* (1968) and Altmann (1972). We have also demonstrated a reduction in labelling and mitotic indices during starvation, confirming the findings of Weibecke *et al.* (1969) in the mouse; however, Hopper *et al.* (1968) were unable to show a decrease in proliferative indices even after 9 days' starvation in the rat. On the other hand Stevens Hooper & Blair (1958) reported a decrease in the number of mitoses per 'crypt section', but this result might be expected on account of the decrease in crypt population.

A reduction in proliferative indices could in principle be caused by a decrease in the growth fraction, that is in the proportion of proliferating cells, or by a decrease in the rate at which cells traverse the cell cycle. We have shown that there is no change in the growth fraction after starvation, and that the reduction in labelling and mitotic indices is due wholly to an increase in cell cycle time. Changes in growth fraction have not hitherto been considered, but we have used the data of Hopper *et al.* (1968) and Rose *et al.* (1971) in the rat, and of Weibecke *et al.* (1969) for the mouse and find no change in the growth fraction as computed from the reported cell cycle parameters and the labelling index. We conclude that changes in growth fraction play no part in the hypoproliferative response evoked in the small bowel mucosa by starvation. This raises the general question of the importance of growth fraction changes in hypoproductive states: upward movement of the 'cut-off position', and hence an increase in growth fraction, has been found during recovery from continuous irradiation (Cairnie, 1967) and in androgen stimulation of the small bowel in the castrate mouse (Wright *et al.* 1972*a*), and this mechanism may augment cell production in hyperproliferative states. Nevertheless, we must not dispense with the growth fraction as a mechanism for reducing cell production, as Wright *et al.* (1972*a*) demonstrated a decrease in growth fraction from 0·65 in control to 0·40 in castrated mice. It is probable that different mechanisms are important in hypoproliferative states in different experimental situations.

Prolongation of the cell cycle time is evidently the mechanism producing the hypoproliferative mucosal state in starvation of the rat, in agreement with the findings of Weibecke *et al.* (1969) in the mouse. We have shown a substantial and significant increase in T_c from 10 hours in controls to 14·7 hours in starved animals. Rose *et al.*

(1971) reported FLM measurements on rats starved for 9 days and, while no real change was found in T_c, a small increase in the duration of the S phase was reported. But this increase was very small, and of questionable importance in the absence of a reliable computer analysis. Furthermore these workers did not report any change in labelling index. However Altmann (1972) has provided some support for the present data by showing an increase in turnover time in rats starved for 7 days. Future work will have to show which phases are responsible for the demonstrated prolongation in T_c; Wiebecke *et al.* (1969) reported increases in all phases, but G_1 was most prolonged.

Upon refeeding the recovery sequence involves a prominent shortening of T_c, from 14·7 to 6·5 hours. Here we have calculated T_c at 16 hours (after refeeding) for comparative purposes only. Fig. 10*b* shows an increased rate of entry into mitosis after refeeding which does not necessarily indicate a reduction in T_c, and may also be explained by an influx of cells from a shortened G_1. We conclude that starvation and subsequent refeeding both involve a modulation of cell cycle time. The small and transient increase in growth fraction noted in the first few hours of refeeding (Fig. 7) is considered to be much less important than the T_c changes in explaining the increased cell production rate. Indeed, at the actual time when cell production rate was measured, i.e. at 16 hours after refeeding, the growth fraction (0·48) was actually lower than that after 96 hours' starvation (0·57).

We have demonstrated an increase in T_c in starvation and a subsequent decrease upon refeeding, but are unable to say with certainty which phase of the cell cycle is predominantly affected. The findings are consistent with a considerable and immediate shortening of t_{g_1}, cells completing DNA synthesis before entering mitosis. Cameron & Cleffman (1964) found a temporal differential between proliferative indices on refeeding starved chicks: such a differential would be consistent with a blockage of cells in G_1, and subsequent release on refeeding. Inspection of the labelling index distribution curve at 96 hours after starvation shows a decrease in the maximum or peak labelling index realized in the proliferative compartment, compared with the peak value after only 4 hours' starvation; also the peak value is again increased after refeeding. Cleaver (1967) noted that, in control animals, the theoretical labelling index as calculated from t_s and T_c is realized in the proliferative compartment, indicating that few, if any, cells are in a resting or G_0 phase within the compartment. Not having measured t_s during starvation and in the initial phases of refeeding we are unable to exclude movement into a G_0 phase within the proliferative compartment after starvation, and later re-entry into the cell cycle with refeeding. Even if this does occur, however, the resting phase here is not a G_0 phase in the strict sense of the term (as applied for example to hepatocytes before entering the proliferative cycle in response to partial hepatectomy), for the labelling index rose immediately after refeeding, with no hint of the pre-replicative period which is common to models of induced DNA synthesis (Stein & Baserga, 1972). We concede that a block in the flow of cells from G_1 to S is possible, and further studies using FLM techniques may settle this question: the changes which have been demonstrated in T_c may be sufficient to account for the observed changes in proliferative indices.

Previous studies on kinetic events after refeeding starved animals have been few,

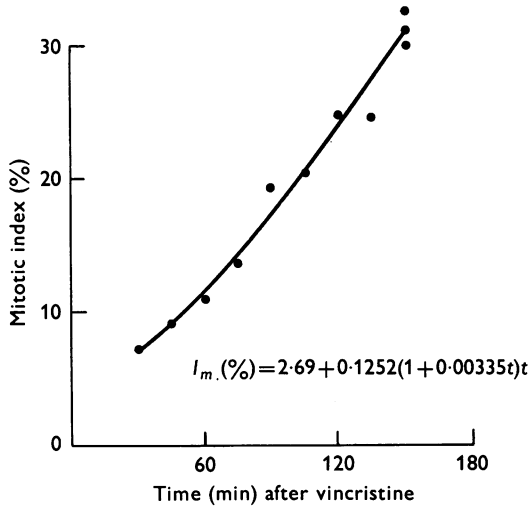

Fig. 12. The data from figure 11(*b*), i.e. the mitotic accumulation line after 16 hours' refeeding, fitted by a quadratic expression.

but Altmann (1972) reported an increase in mitotic index after refeeding rats starved for 7 days; there was also a decrease in turnover time within 24 hours, in accord with the present observations. Altmann failed to find any increase in crypt size after refeeding, although there was an increase in villous size.

The changes in cell cycle time were accompanied by modifications in migration rate and transit time (Table 1). The migration rate was decreased after starvation, which agrees with the measurements of Hopper *et al.* (1972) and of Weibecke *et al.* (1969) made by observing the migration of villus cells labelled with ^{3}HTdr.

We have already alluded to the difficulty in interpreting mitotic accumulation results in a dynamic situation where the mitotic index is itself increasing. In the absence of changes in growth fraction and mitotic duration, any acceleration of mitotic accumulation during the stathmokinetic period must be due to a reduction in T_c. An appreciation of this point is possible from inspection of Fig. 12, which shows the data from Fig. 10*b* fitted by a quadratic expression; the fit is obviously good, and may be preferable to a linear fit, though no better than would be expected with an extra parameter. A tangent drawn to the curve at any point is equal to the rate of entry into mitosis at that point. For comparative purposes we have used a linear model in Fig. 10*a*, and the slope of the regression line in the refed animals is significantly greater than that in the starved animals. Because of the shorter time factor, stathmokinetic experiments are preferable to longer term labelling experiments in situations where cell cycle parameters are changing rapidly. Against this must be balanced the less complete form of the data, and the possibility of changes in the rate of entry into mitosis even over so short a period as 2·5 hours.

In conclusion, we have found that cycle parameter changes are the chief means whereby the hypoproliferative response to starvation is produced in the rat small intestinal mucosa, and changes in T_c maybe more important in crypt kinetic reactions

than previously realized (Wright *et al.* 1972*a*), particularly in hypoproductive states. Furthermore Lesher & Bauman (1969) have reported a reduction in T_c from 12 to 7·5 hours in the small bowel of irradiated rats, and Wright, Watson, Morley, Appleton, Marks & Douglas (1973) have recently demonstrated a reduction in T_c in patients with coeliac disease. Cell cycle time changes may also emerge as important factors in increases of cell production.

SUMMARY

Male rats were starved for a period of 96 hours. Measurements of crypt cell population showed a small reduction during starvation. The growth fraction remained constant, but the total number of proliferating cells per crypt fell as a consequence of the reduction in crypt population. Both labelling and mitotic indices fell throughout the starvation period. The cell cycle time (T_c), measured by a stathmokinetic technique using vincristine, was increased from 10·4 hours in control rats to 14·7 hours after 96 hours' starvation.

Upon refeeding, the proliferative indices were observed to rise. After a small initial fluctuation, the growth fraction remained constant. The crypt population remained substantially unchanged. Sixteen hours after refeeding, the cell cycle time was reduced to 6·5 hours.

The hypoproliferative response to starvation is mediated solely by an increase in cell cycle time, and the response to refeeding is interpreted in terms of a reduction in T_c. Changes in the size of the proliferating population are considered not to play an important role in either response, although it is not possible to exclude entirely the presence of resting cells in the proliferative compartment itself.

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