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BEHAVIOUR OF THE PROLIFERATIVE COMPARTMENT OF THE SMALL INTESTINE AT DIFFERENT TIMES OF THE DAY

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Many questions concerning the kinetics and functions of the epithelial cells—the columnar cells in particular—have still to be clarified, despite several investigations on the small intestine.

The anatomic structure of the small intestine makes it easy to distinguish the proliferative compartment, the Lieberkühn crypts, from the functional compartment, the villus. The small intestine appears, therefore, to be one of the most suitable tissues for the evaluation of the effects of chemical and physical agents on its steady state.

In the crypt-villus junction the cellular differentiation takes place by which the proliferative cells lose their ability to divide and synthetize specific enzymes such as disaccharases, dipeptidases and other molecules localized in the plasma membrane of the microvilli. These enzymes carry out the membrane digestion process (EICHHOLZ & CRANE 1965, DAHLQUIST & NORDSTRÖM 1966, FORTIN-MAGANA et coll. 1969, JAMES et coll. 1971, UGOLEV 1972). They hydrolize di- and tri-saccharides and di- and tri-peptides, and the corresponding monomera are then absorbed through the specific mechanism of active or passive transport. The recently detected existence of circadian oscillations in the activities of the brush border enzymes (SAITO et coll. 1975, STEVENSON et coll. 1975, BECCIOLINI et coll. 1977, SAITO et coll. 1978), has made the system even more complex.

The present investigation was made to find out whether animals killed at different times of the day would show circadian variations in the mitotic and labelling index and mainly in the distribution of labelled and mitotic cells along the crypts.

Material and Methods

Forty-eight female Wistar rats 10 to 12 weeks old and weighing 180 to 200 g were used. The animals were kept under constant conditions with an L/D cycle from 6.30 a.m. to 6.30 p.m., food and water ad libitum. The cages were always cleaned and the rats always fed early in the morning by the same person every other day.

Six groups of 8 or 9 animals each were killed at 0, 4, 8, 12 a.m., 4 and 8 p.m., within a maximum time of 50 min. Six animals from each group were injected with 3.7 GBq (100 μCi) of 3H Thymidine specific activity 74 GBq/mmol (2 Ci/mmol) 1 h before being killed.

Immediately after the death the small intestine was removed, opened longitudinally, washed many times in cold 0.9 per cent NaCl, and cut into 5 equal parts for enzyme activity assay. One cm long pieces were cut at the end of the first and third segments (proximal and distal jejunum). The results for the proximal jejunum are now reported.

Other experimental conditions such as the treatment of the small intestine and the assay of enzyme activity have been reported previously (BECCIOLINI et coll. 1982 b). Invertase activity was assayed ac-

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cording to DAHLQUIST (1964) and the activity was expressed as U/g of protein. The histologic and autoradiographic techniques have been reported previously (BECCIOLINI et coll. 1982a). In the autoradiographic sections the background was very low.

When the labelling was too strong and capable of affecting the mitosis counts, these were carried out on sections without emulsion. Late prophase, metaphase and anaphase were counted for the mitotic index and mitosis positions.

The labelled and mitotic cell distribution was determined by recording as number 1 the first cell on the left of the crypt-villus axis. Only the cells whose nuclei were visible in the section and whose cytoplasms were touching the basement membrane were counted. The border line between crypt and villus is not exactly defined but identification is made possible by the different morphology of the nuclei and cell dimensions and by the presence of a knee where the crypt joins the villus.

Fifty and eighty crypt-villus formations were counted for the distribution of labelled and mitotic cells, respectively. The mean values of the count for each animal were used to calculate the mean ±SE for the animals in each group.

As it would have been meaningless to compare the same position of labelled and mitotic cells in different crypt-villus formations because of the different number of epithelial cells, the heights of the crypts were normalized. In this way the area covered by each cell was proportional to the number of cells of the proliferative compartment. The data from the counting were grouped first with reference to individual animals, then to groups, and were processed by a program in Fortran language.

In the reconstructed crypt, the positions occupied by the goblet, enteroendocrine and Paneth cells were not taken into account as these cells are few and they were present in a constant number in control animals killed at different times of the day.

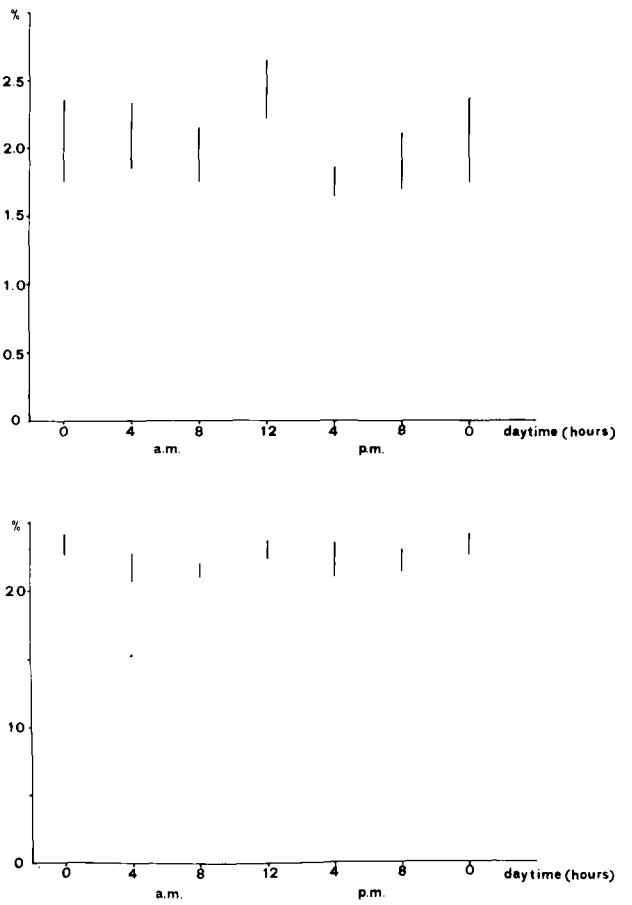

Fig. 1. Mean values ±SEM of mitotic (upper part) and labelling (lower part) indices in the groups of animals killed at different times of the day.

Results

The microscopic examination revealed no alterations either in the epithelium or in the stroma. The number of the cells in the crypt and in the crypt-villus formation differed slightly in the single animals and among the animals in the different groups. The highest and lowest values of these parameters in the proximal jejunum among the different groups varied between 37.56±0.76 and 38.90±0.41 and between 107.61±3.18 and 115.66±1.33, respectively, for the crypt and for the whole formation.

The labelling index (Fig. 1) does not present marked differences among the animals killed at different times of the day: the values varied between 21.5 and 23.5 per cent. The highest levels of 3H Thymidine uptake were observed at midnight and at noon.

The mitotic index at the different times of the day (Fig. 1) demonstrated no marked differences. Two peaks, during the night and at 12 a.m., were observed. The differences between the values in the 12 a.m. group and those in the 8 a.m., 4 p.m. and 8 p.m. groups are statistically significant ($p<0.01$).

Labelled cell distribution. The frequency of labelled cells along the crypt appears in Figs 2 and 3. The curve is continuous but preferential localizations occurred.

At the bottom of the crypt the frequency of la-

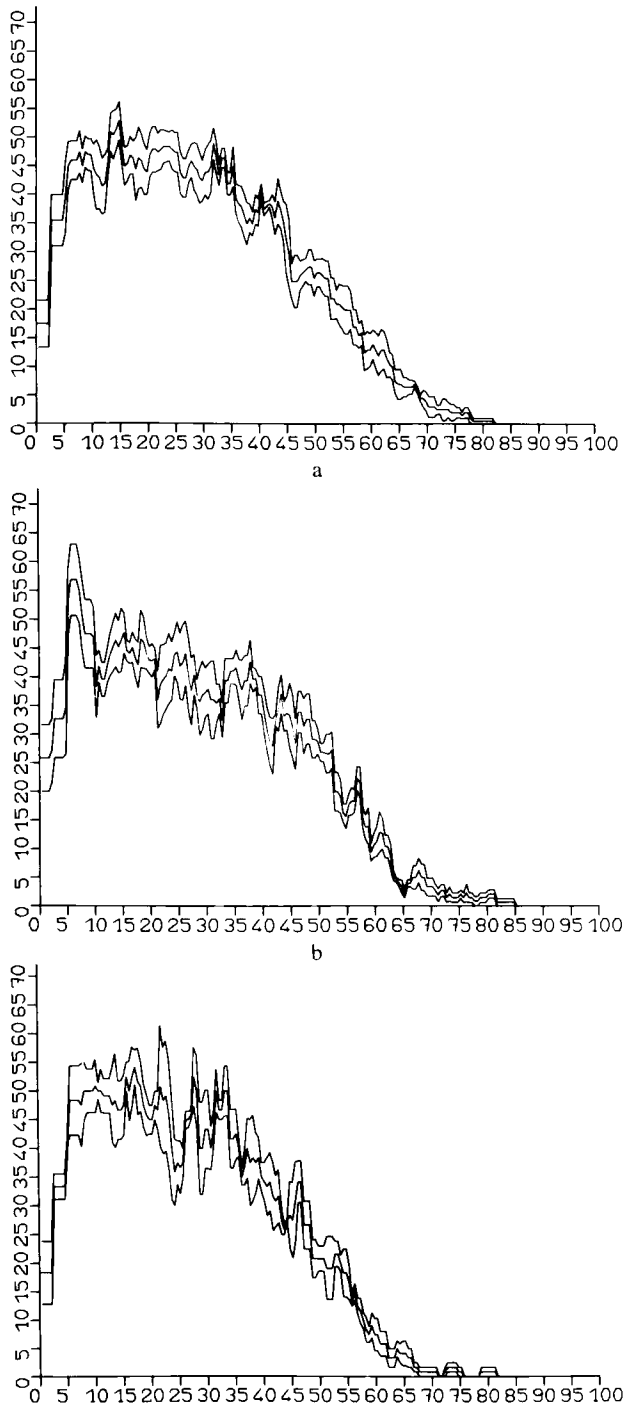

Fig. 2. The frequency, as per cent of labelled cells between the highest and lowest limits of SEM in the theoretic crypt of animals killed at midnight, 4 a.m. and 8 a.m. The mean number of epithelial cells in the left side of the crypt was 38, 39 and 38, respectively.

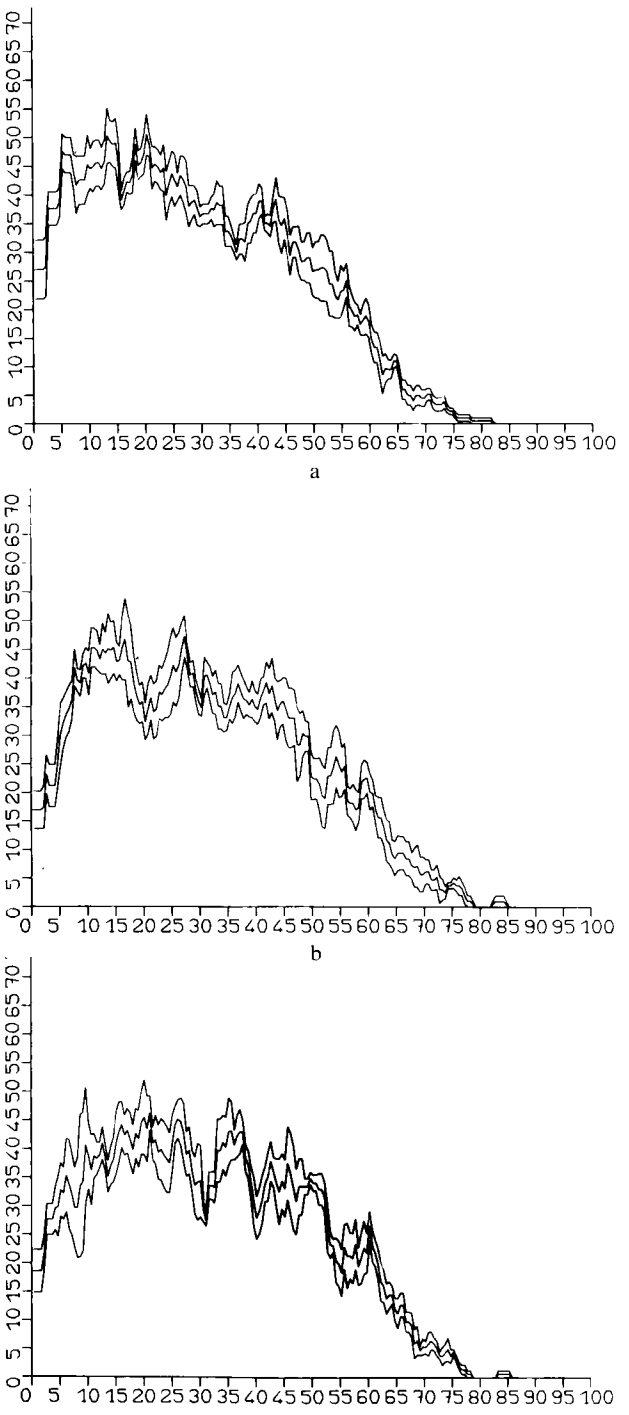

Fig. 3. The frequency, as per cent of labelled cells in the theoretic crypt of animals killed at noon, 4 p.m. and 8 p.m. (cf. Fig. 2). The mean number of crypt cells was 39, 38 and 39, respectively.

belled cells was low, then increased rapidly until the highest frequency (about 50 per cent) was reached in the lower third. This value decreased more or less gradually, in the various groups, in the middle third and reached levels of about 5 per cent. Labelled cells were few in the upper part of the crypts and they were absent in the upper 20 per cent.

Some important differences were observed be-

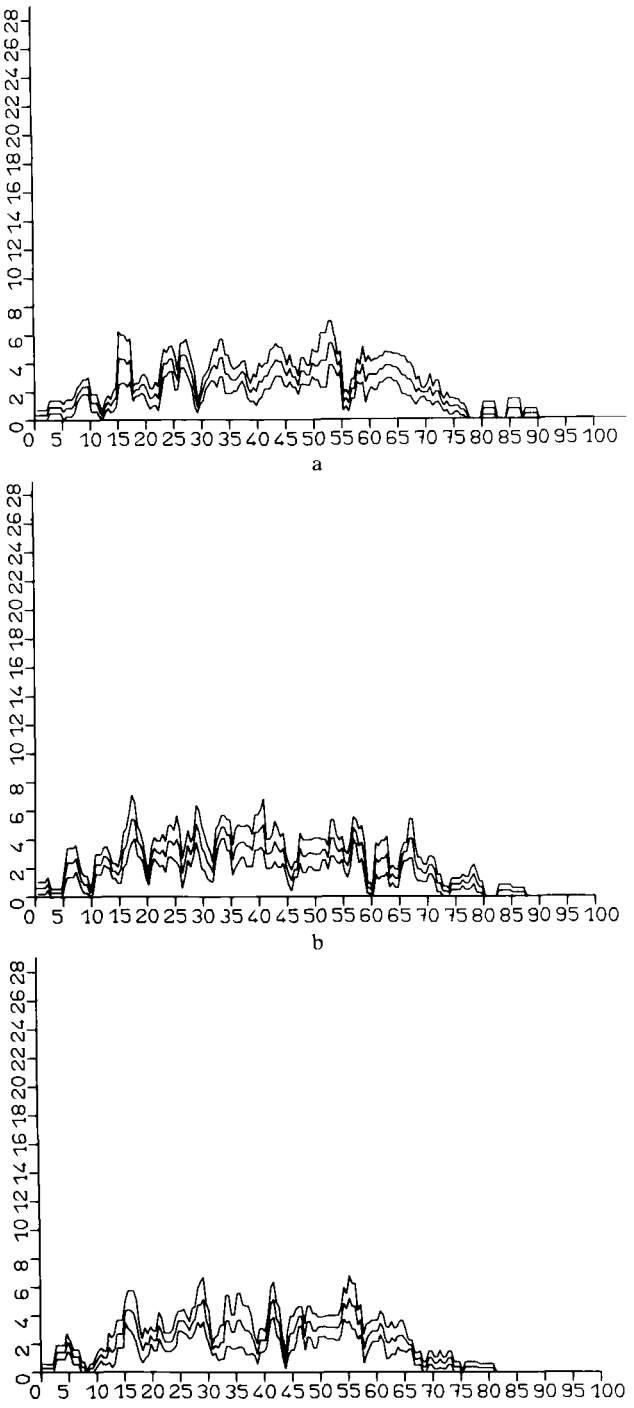

Fig. 4. The frequency of mitoses between the highest and lowest limits of SEM in animals killed at midnight, 4 a.m. and 8 a.m. The values refer to the single cell positions in the computed theoretic crypt. The mean number of crypt cells was 38, 39 and 38, respectively.

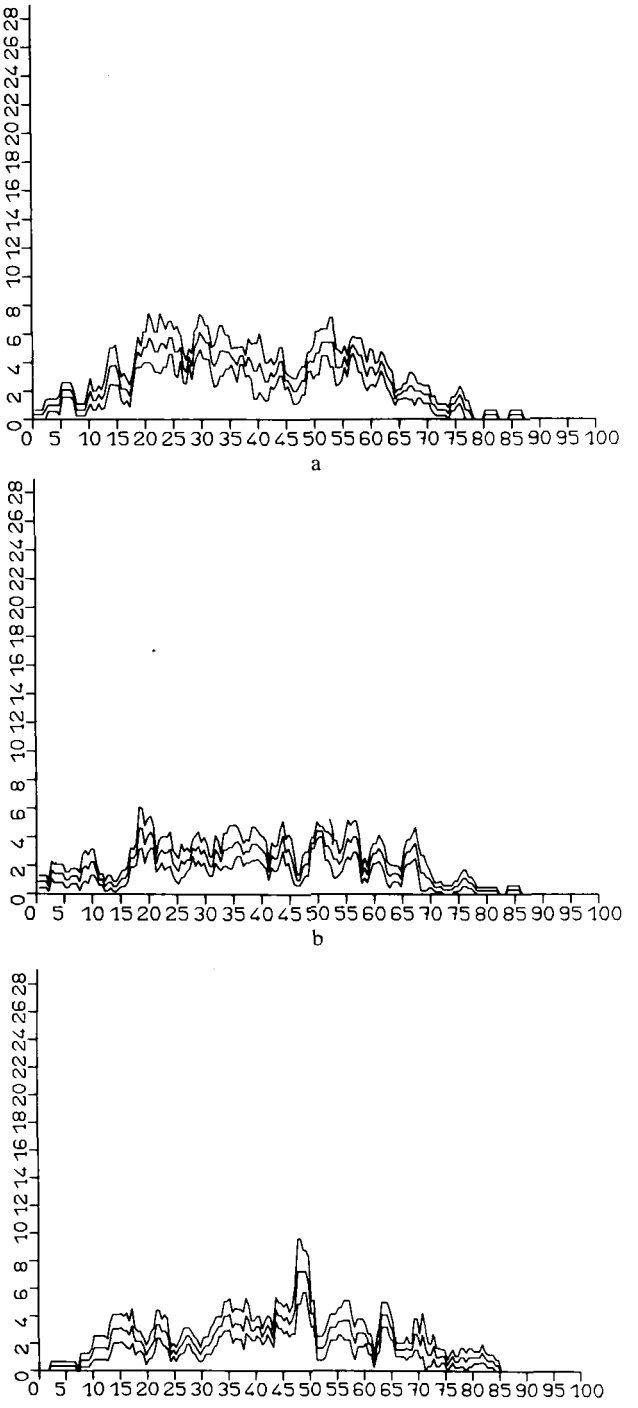

Fig. 5. The frequency of mitoses in animals killed at noon, 4 p.m. and 8 p.m. (cf. Fig. 4). The mean number of crypt cells was 39, 38 and 39, respectively.

tween the groups of animals killed at different times of the day. The labelled cell distribution at the 0 interval was similar to that at the 4 a.m. interval. At 8 a.m. the frequency was over 50 per cent in the lower third and the values were lower in the middle third than those of previous intervals.

The frequency of labelled cells in the initial third was lower in the group killed at 12 a.m. while it

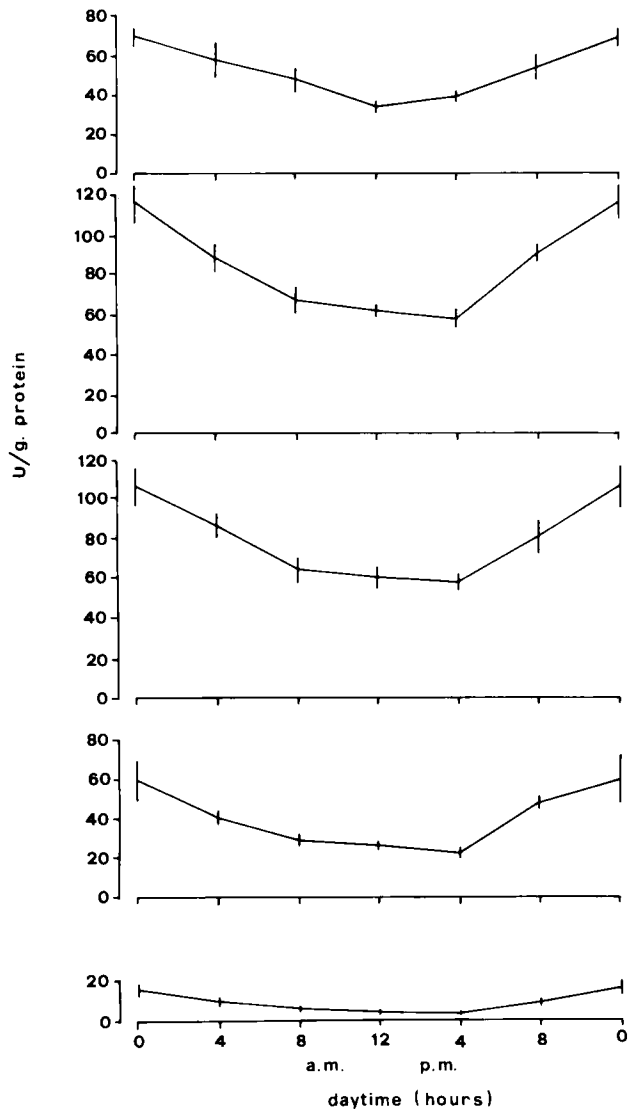

Fig. 6. Invertase activity in the 5 tracts of the small intestine at different times of the day.

increased in the middle third. This occurrence was more marked in the 4 p.m. group and particularly in the 8 p.m. group when the labelled cells appeared homogeneously distributed in the lower half of the crypt with values lower than at the other intervals. The labelled cell frequency, in the lower and in the upper parts of the crypt, was significantly higher than in the other groups ($p<0.02$). The greatest differences were evidenced between the 8 a.m. and the 8 p.m. groups.

Mitotic cell distribution. The proliferative rhythm in the small intestine is high but the number of mitoses is not sufficient for an assessment of preferential localization along the crypt. Although 80 crypt-villus formations for each animal were counted, the mitosis frequency in every position appeared very low and therefore highly variable among the animals, nevertheless the differences in the distribution along the crypt were evident (Figs 4, 5).

In all the groups of animals killed at different hours the mitosis frequency was close to zero in the first position, and it was also low in the initial 5 positions.

The distribution of mitotic cells was rather homogeneous between 15 and 65 per cent of the whole crypt, and reached the highest frequency levels of about 6 per cent.

Although some of the mitoses were located in the upper third of the crypt, they were absent in the highest 10 per cent.

The different groups behaved similarly although a higher frequency of mitoses in the area covering 20 to 60 per cent of the whole crypt was observed in the animals killed at 12 a.m.; the frequency in the lower half of the crypt decreased significantly in the group killed at 8 p.m. ($p<0.02$).

Brush border enzyme activities. Invertase activity, assayed in the 5 tracts (Fig. 6), demonstrated a clear circadian oscillation in all the tracts with the highest values at 0 h and the lowest between 12 a.m. and 4 p.m. The oscillations were more marked in the central tracts where the activity was higher.

Discussion

The existence of bioperiodical phenomena in the function and kinetics of the epithelial cells in the small intestine has been investigated to collect data for a possible future clinical use. The same doses of antineoplastic agents can have, both pharmacologically and at radiation therapy, different biologic effects depending on the hours when they are administered. The present data demonstrate that the values of proliferative and mainly of functional activity in the small intestine show statistically significant differences during the day.

Two peaks in the mitotic and labelling indices were observed in the night and at noon. This result can be explained by the fact that the cell cycle time is about 10.5 h, with varying lengths between the bottom of the crypt, where it is longest, and the top of the crypt. The oscillations of the labelling index did not appear so marked because the length of the S-phase is about 60 to 70 per cent of the total cycle time (CAIRNIE et coll. 1965).

SIGDESTAD et coll. (1969) and SIGDESTAD &

LESHER (1970), using squash, microcolony techniques, demonstrated a circadian dependence of the proliferation in the small intestine which reached its highest levels during the night and its lowest in the daytime. When histopathologic techniques were used, different results were obtained (AL-DEWACHI et coll. 1976, KLEIN 1980). PILGRIM et coll. (1963) observed that the mitotic index in the crypts of the jejunum is high during the night and in the middle of the day, the lowest values being reached at 5.30 p.m. ALOV (1963) was of the opinion that the continuous feeding in animals which have free access to food either in the daytime or at night can limit the oscillations of the mitotic index.

The distribution of labelled and mitotic cells along the crypt differed, and the mitosis localization always appeared shifted to positions higher than that of the labelled cells. In fact during their turnover, the cells pass their cycle.

Current data confirm that, when a cell reaches a certain position in the crypt, it loses its ability to proliferate and begins to synthetize the brush border enzymes through the differentiation process. Therefore, a region varying in length exists in the proliferative compartment where the cell 'decides' whether it is possible to begin a new mitotic cycle or the differentiation process (CAIRNIE et coll.). A mitotic cycle will start only if it can be concluded within the space and the time evidenced by the distribution of labelled and mitotic cells.

At present, mathematical models are being analysed to see if a relationship exists between the frequency of labelled cells and of the mitoses in the different positions and also to investigate the temporal sequence of mitotic cycles in the crypts.

The distribution of labelled cells in the crypts appeared different in the animals killed during the day. The S-phase cells are more frequent in the upper half of the crypt during the intervals at the end of the light period and early in the dark period. This should lead to a reduction in the differentiation area and consequently to a decrease in the synthesis or activity of the enzymes. Since these enzymes are also present in the villus cells, this phenomenon would contribute only slightly to the circadian oscillations of the activities of the brush border enzymes.

The correlation between these parameters does not appear to be close, as, from a functional point of view, the enzyme activities localized in the villus are quantitatively more important and the synthesis or activation of the brush border enzymes can occur also during the villus cell migration. On the other hand, the epithelial cell turnover in the crypt-villus system is longer than an L/D period. Therefore the circadian oscillation of the activities occurs more than once during the life span of the cell.

The different distribution of the proliferative cells in the phases of the cell cycle at different times of the day leads to the evaluation of the effects of ionizing radiation on the intestinal epithelium. The aim of these forthcoming experiments is to try to induce less heavy cellular injury, leading to the most rapid recovery after the same dose given at varying hours during the day.

SUMMARY

The modifications of mitotic and labelling indices in animals killed at different hours of the day were analysed. The invertase activity was also assayed. This brush border enzyme; synthetized by the differentiating cells, showed a clear circadian rhythm with a maximum in the night and a minimum near the end of the light period. Mitotic and labelling indices showed the highest activity in the night and at noon. The positions of mitotic and labelled epithelial cells in the crypt were also determined. The frequency of labelled cells in the different positions of the crypt evidenced a shift of these cells towards the crypt-villus junction in the late afternoon with a consequent reduction of the differentiating compartment.

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