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IRRADIATION AT DIFFERENT TIMES OF THE DAY

Morphology and kinetics of the small intestine

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The influence of bioperiodical phenomena on functional and proliferative activity in tissues with different cellular turnover (BULLOGH 1948, PILGRIM et coll. 1963, SIGDESTAD 1969, SIGDESTAD & LESHER 1970, FURUYA & YUGARI 1974, SAITO et coll. 1975, BECCIOLINI et coll. 1977 b, KLEIN 1980, BECCIOLINI et coll., in preparation) suggested that exposure to ionizing radiation at different times of the day may be capable of modifying post-irradiation effects.

In previous experiments the irradiation was generally carried out late in the afternoon and the time of exposure was not rigidly fixed.

Morphologic and kinetic parameters of the small intestine in rats, previously irradiated with doses of 2 to 20 Gy (BECCIOLINI et coll. 1972, 1973, 1974, 1976, 1977 b, 1979), were analyzed to obtain as much information as possible. Biochemical research concerned the modifications in the activity of the brush border enzymes, synthetized by the epithelial cells during the differentiation process, and the modifications of lysosomal enzymes as indices of cell injury. The previous experiments had evidenced a characteristic behaviour of brush border enzymes at the onset of injury to the epithelial cells (BECCIOLINI et coll. 1972, 1973, 1974, 1976, 1977 a, b, 1979).

The aim of the present experiments was to evaluate whether, by irradiating the animals with the same dose at different functional moments, it was possible to differentiate the onset of cell injury through qualitative and quantitative morphologic investigation.

Materials and Methods

The series consisted of 368 female Wistar rats, 10 to 12 weeks old, weighing between 180 and 200 g. The animals were kept, before and throughout the experiment, under constant conditions with a 6.30 a.m. to 6.30 p.m. L/D cycle and food and water ad libitum. The cages were cleaned early in the morning by the same person every two days. The animals were divided into 4 groups: group A was irradiated at 0, B at 6.00 a.m., C at 12.00 and group D at 6.00 p.m. The rats were exposed over an abdominal area of 5 cm×5 cm, including the whole small intestine, with 8 Gy from a telecobalt unit. The irradiated animals were killed in groups of 5 at 6, 12, 36, 48 and 72 h after irradiation to analyse the early phases of radiation injury. In order to assess the return to normal appearance of the epithelium, groups of animals were killed at 96, 120, 126, 132, 144 and 150 h, 11, 20 and in groups C and D 29 days after irradiation. Six groups of 8 animals each killed every 4 h during the L/D cycle were used as controls. Three animals from each irradiated group and 6 from each control group were injected with 100 μCi of H^3Thymidine (specific activity: 74 GBq/mmol, 2 Ci/mmol). Both irradiation and killing were carried out within a maximum time of 50 min. Immediately after death the small intestine was removed, longitudinally opened, washed in cold 0.9% NaCl and cut into five equal parts for enzyme activity assays. A one cm long piece cut at the end of the first segment (proxi-

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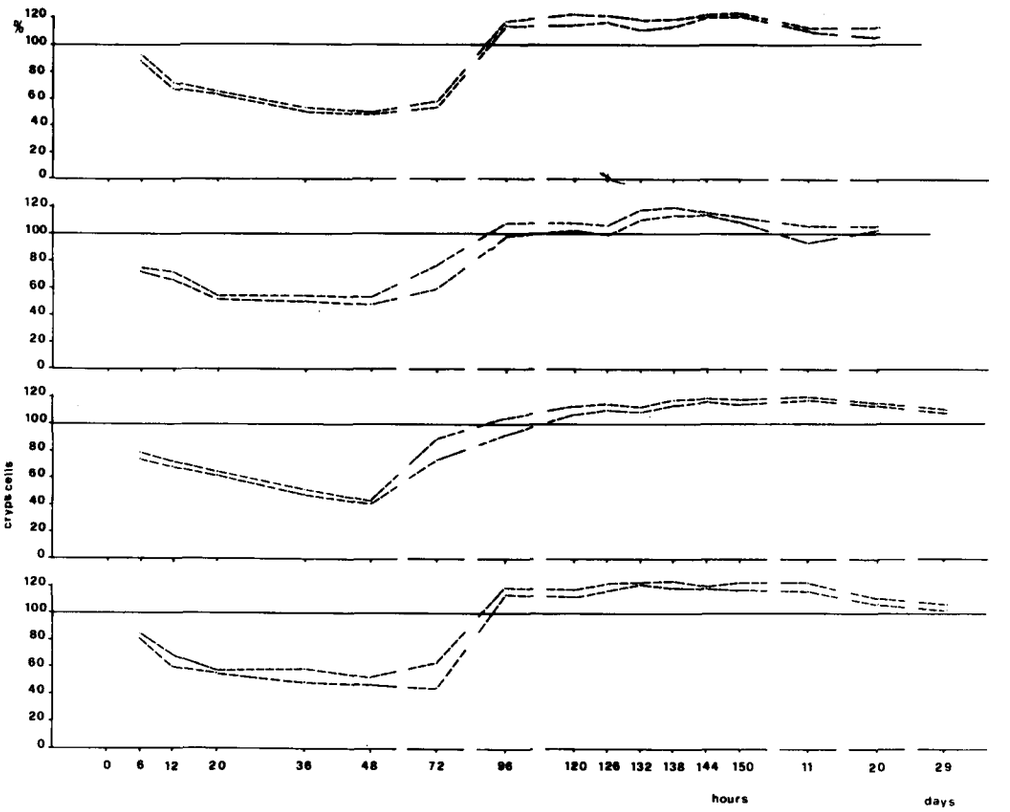

Fig. 1. Mean number ±SE of crypt cells in the animals irradiated at 0, 6, 12 a.m. and 6 p.m. expressed as per cent of controls is reported versus the post-irradiation time. Different groups are represented from top to bottom as broken lines; control values are represented as a band.

mal jejunum) was used for microscopic examination. In order to have well-aligned crypt-villus formations these segments were spread on a thin pasteboard slab with the muscular tissue stuck to it. The segments were fixed using Carnoy's solution. An Ilford K5 emulsion was used for autoradiography; the sections were then stained with hematoxylin-eosin.

For quantitative morphologic examination only the formations longitudinal to the villus axis were counted by 3 experienced persons. For each animal 45 counts at least were carried out beginning from the first cell on the left of the crypt-villus axis. The number of epithelial cells of the crypt-villus system and also labelled cells and mitoses were counted. The nuclei with a number of Ag grains 8 to 10 times higher than the background, observed in an area of similar size, were considered labelled. Late prophases, metaphases and anaphases were counted for the mitotic index.

The number of epithelial cells in the left side of the crypts and in the whole formation showed statistically insignificant variations in control animals killed at different times of the day; therefore, all data were averaged.

The values of total and crypt cells were expressed as per cent of controls.

The counts from each animal were used to calculate the mean±SE for every group killed at the different intervals. Student's t-test was used for statistical evaluation.

Results

Morphologic observations. Six hours after irradiation alterations were confined to the lower part of the crypt; Feulgen positive fragments and some enlarged nuclei were observed, they were not so marked in group A.

At 12 h the crypt cell nuclei, as high as the crypt-villus junction, appeared irregular in shape and size, misaligned and spaced out. Mitotic activity was always mild. Villus cells appeared normal. In group A alterations were more marked than in the other

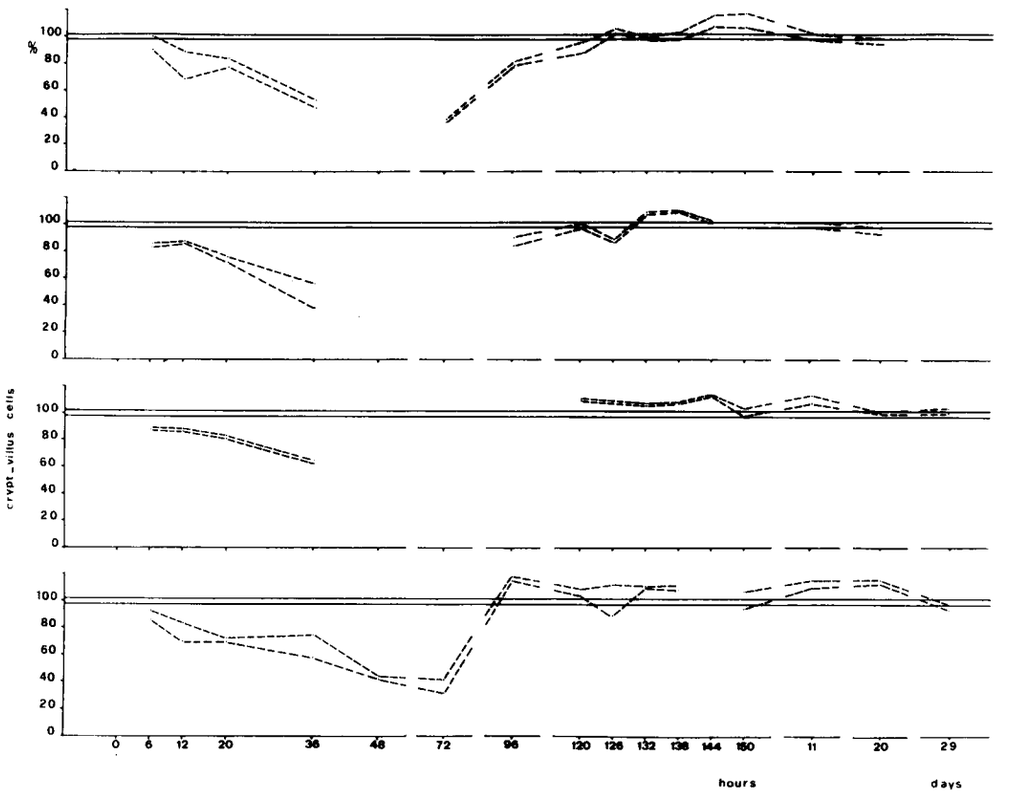

Fig. 2. Mean total number ±SE of crypt-villus cells in the different groups of irradiated animals (cf. Fig. 1).

groups. At 20 and 36 h the progressive decrease in epithelium height was very marked. Loss of organization in the crypts and the presence of phlogosis cells in the stroma were observed in all irradiated groups. Cell alterations involved the two lower thirds of the villi at the 36 h interval. The crypt-villus systems 48 h after irradiation appeared highly reduced in height with disorganized crypts and conglutinated villi; injured cells were present in the whole formations and vacuolated cells were observed at the top of the villi. Non-structured areas, stroma very rich in phlogosis cells and regenerating crypts, which were high if compared with the rest of the formation, were observed at 72 h. In these areas many cells were still altered; however, the crypts were again aligned and mitotic activity was considerable. A thin epithelium covered the villi with cells often without nucleus and vacuolated. Marked differences were observed both in different areas of the same section and among the animals of the same group. Recovery in groups B and C seemed to be more advanced.

In the animals killed after 96 h differences in recovery were evident in all irradiated groups. In some sections unstructured areas prevailed, with enlarged vases and villi which were greatly reduced in height, in other areas regenerating crypts and numerous mitoses were frequent.

In the 120 h group crypt-villus formations appeared to be well structured with cells which were nearly normal, apart from some globous nuclei at the top of the villi.

At 126 to 150 h the morphology of the epithelium appeared similar to the controls, although in a few areas recovery was still going on. The morphology of the small intestine epithelium appeared completely normal 11, 20 and 29 days after irradiation.

Number of epithelial crypt cells. The values of the crypt cells in the different control groups varied between 37.56±0.76 and 38.90±0.48. For the whole formation the epithelial cells ranged between 107.61±3.18 and 115.66±1.33.

In all irradiated groups a significant decrease in the epithelial cells of the crypts was observed from the first interval after exposure (Fig. 1). Later, values decreased progressively and reached the lowest

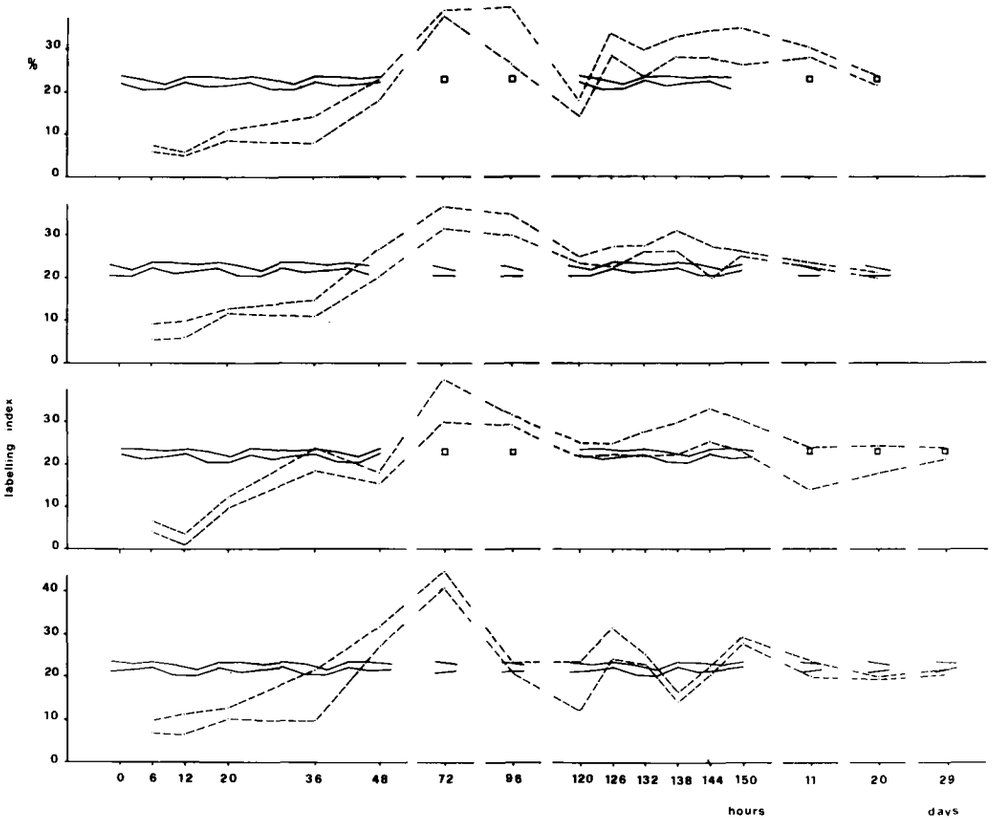

Fig 3. Labelling index ±SE in controls (continuous line) and in different groups of irradiated animals (broken line; cf. Fig. 1).

levels—about 50 per cent of controls—after 48 h. From this interval to 96 h the epithelium appeared so damaged that only some areas were counted. Values in these groups must be regarded as representative of only those areas where some organization of the epithelium was present.

On the whole the groups of animals irradiated during the L/D cycle showed similar behaviour. However, some differences were evidenced: in the rats exposed at the end of the light period and during the night (groups D and A) the initial decrease was not so marked as in the other groups. The curves of crypt cell reduction in these two groups were parallel until 48 h. Values in group C decreased steadily up to 48 h, when they were lower than in the other groups. After 72 h epithelial cells increased in all groups, especially in groups B and C.

It is worth noting that the number of cells increased well above controls in the well structured areas of groups A and D 96 h after exposure. At later intervals the number of crypt cells was significantly higher than in controls ($p<0.02$). In group B the same behaviour was observed at some intervals only, and control values were reached as early as 11 days after exposure.

Total number of epithelial cells in individual animals and groups was more markedly variable than that of crypt cells. Sometimes it was impossible to count as many as 40 crypt-villus formations, particularly after radiation injury had set in. Therefore, at 48, 72 and 96 h after exposure the counts in some of the groups were too scarce and thus not reported in Fig. 2.

In all groups the number of cells decreased progressively according to the time after irradiation. At 36 h the decrease in the number of cells in groups A and B was significantly higher than in the other two groups.

In those groups where counts were possible, the lowest levels were reached at 72 h. Then an increase in epithelial cells was observed and at some of the intervals the values were higher than in controls. This increase was less marked than that in crypt

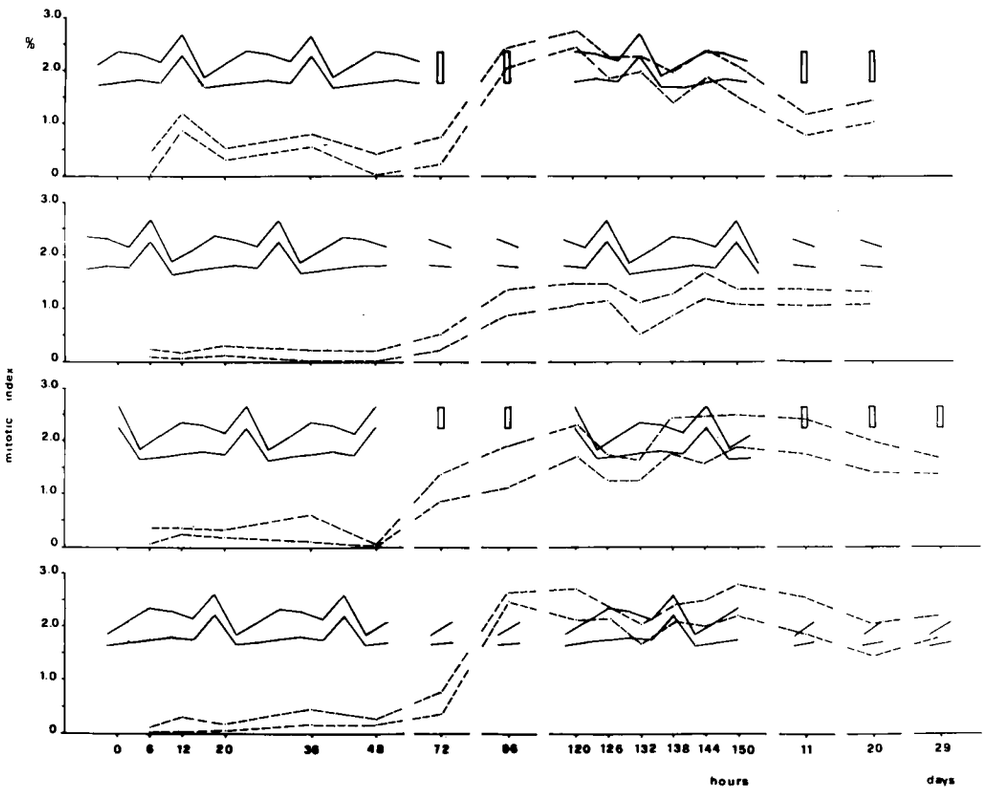

Fig. 4. Mitotic index ±SE in controls (continuous line) and in different groups of irradiated animals (broken line; cf. Fig. 1).

cells and generally its values were similar to controls at the last intervals.

Labelling index evidenced variations in the control animals killed at different times of the day. The highest levels were observed at 0 h and at 12.00 a.m. The differences between highest and lowest values appeared to be statistically significant ($p<0.02$). Therefore, values from control animals killed at the same time of the day were reported above each group (Fig. 3).

As early as 6 and 12 h after exposure in all groups the number of cells capable in incorporating H^3Thymidine decreased markedly and the labelling index was 30 per cent of control values. The number of labelled cells in group C was particularly reduced after 12 h. At 20 and 36 h, although the number of labelled cells was still reduced due to the reduced number of crypt cells, the labelling index increased but was still significantly lower than in controls with the exception of group C at 36 h ($p<0.01$ in groups A and B).

In the animals killed 48 h after exposure the labelling index increased although values from the different groups varied significantly except for groups C and D. The number of labelled cells at this interval was about 50 per cent of controls.

At 72 h the labelling index increased significantly in relation to that in controls and it was more marked in groups A and D.

At 120 h the labelling index was equal to or actually lower than in controls in all groups; it then increased again and persisted at significantly higher levels until 20 days in group A and until 11 days in the other groups. Group B showed values more similar to those in the controls.

Mitotic index also varied according to the time of the day when control animals were killed. The highest value was observed at 12 h but levels were high also during the night.

Control values (Fig. 4) correspond to the hours when irradiated animals were killed.

Up to 48 h the mitotic index had values close to zero in all groups except group A. Starting from this interval the mitotic index gradually increased and at 96 h it reached control values (group A) or higher ones (group D). In groups B and C the levels were still significantly lower. At subsequent intervals the mitotic index of group B remained significantly low-

er than in controls throughout the experiments. In the other groups the levels were similar to those in non-irradiated animals.

It is worth noting that in this phase the values at different times of the day did not coincide with the circadian oscillations of controls. The mitotic index was significantly lower than in controls even in group A at 11 and 20 days and in group C at 20 and 29 days.

Discussion

The aim was to evaluate the quantitative and qualitative morphologic modifications on the small intestine when the abdomen was irradiated at 4 different times of the day. The general behaviour of morphologic alterations appears to be similar to that previously observed (QUASTLER 1963, GALJAARD & BOOTSMA 1969, BECCIOLINI et coll. 1972, 1973, 1974, 1976), and no substantial differences were observed among the irradiated groups, apart from group A where less marked modifications at the early intervals seemed to exist. Cell alterations, which were initially localized in the proliferative compartment only, extended later to the differentiated compartment. Subsequently, because of turnover, the injured cells appeared also in the functional compartment and the epithelium appeared flattened due to the block in proliferative activity. During recovery no marked morphologic differences were observed in the 4 irradiated groups.

The number of epithelial cells in the left side of the crypts in groups D and A, irradiated at the end of the light period and at midnight, respectively, showed a smaller reduction at the first killing interval. In these groups recovery was slower and at 72 h the number of cells was similar to the lowest values recorded at 48 h.

It is worth noting that 96 h after irradiation a significant increase was observed in groups A and D whereas groups B and C increased later. Group B, irradiated at the end of the dark period, presented a number of cells more similar to that in controls starting from 96 h; this was the only group in which control values were reached 11 days after irradiation.

The number of crypt-villus epithelial cells in all groups was reduced at the early intervals caused by cell death and then by the lack of mitoses; later the turnover led to a significant decrease (less than 50%) in villus cells.

At later intervals the high crypt proliferation affected the epithelium, but only at some intervals was the total number of cells higher than in controls.

The behaviour of the labelling index was a clear indication of post-irradiation injury and recovery. The injury in the proliferative compartment appeared clearly at the early intervals when the number of cells which were able to incorporate H^3Thymidine was three times as low as in controls. At later intervals the reduced number of crypt cells led to an increase in the labelling index and at 72 h this parameter went up significantly due to active proliferation. Obviously values were higher in those groups where the number of crypt cells was still markedly reduced.

The increase of crypt cells leads to a reduction of the labelling index: this result justifies the recovery of the cell differentiation process with the synthesis of brush border enzymes (BECCIOLINI et coll. 1982). A significantly increased labelling index was present even at some of the following intervals, but 11 days after irradiation, or 20 days in group A, the levels of controls were reached.

During this phase group B behaves similarly to controls.

As for the post-irradiation behaviour of the mitotic index a block in proliferative activity was evidenced in all groups exposed at different hours and it persisted even at 72 h when labelled cells had markedly increased. Subsequently, values went back to control levels except for group B where the mitotic index appeared to be significantly lower.

During recovery the mitotic index in the animals killed between 120 and 150 h varied again but it did not parallel control behaviour.

It is interesting to note that, in group B and at the latest intervals in the other groups, despite a reduced mitotic index, the number of cells in the crypt and in the whole formation did not appear to be reduced. This may indicate a different steady state.

On the whole, the present results demonstrate that by irradiation at different hours no morphologic injury was evidenced which could be regarded as drastically different. On the other hand the dose was quite high and cell injury may have been so severe as to exclude a possible greater differentiation in the post-irradiation effects among the groups.

The dose administered was not high enough to lead to radiation intestinal death and consequently no survival analysis was possible.

However, the differences observed were quantitatively important and they seemed to indicate a light-

er early injury in the groups irradiated at the end of the light period and during the night, whereas the injury induced by the exposure at the end of the dark period seemed to be more easily recovered. In the latter case the number of epithelial cells and the labelling index in the intestinal epithelium went back to control values more rapidly, although the mitotic index persisted on levels lower than in control animals.

SUMMARY

Rats were irradiated at different times of the day with sublethal doses on the abdomen only, and qualitative and quantitative morphologic modifications were determined. The experiments seemed to demonstrate that in the groups irradiated at night and at the end of the light period early injury is not severe whereas in the group irradiated at the end of the dark period repair of the injury seems to be more effective.

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