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CELL PROLIFERATION AND DIFFERENTIATION IN THE SMALL INTESTINE AFTER IRRADIATION WITH MULTIPLE FRACTIONS

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Abstract

Qualitative and quantitative morphologic changes in rat small intestine were studied after abdominal exposure to multiple fractions of gamma radiation. One group of animals received 3×2 Gy with one fraction every 4 hours. Another group received two courses of this type with a 16 hour interval between the courses (total dose 6×2 Gy). A marked decrease in the number of crypt epithelial cells, and in mitotic and labelling indices, was observed up to 24 to 36 hours after the end of both regimens. Repair and recovery occurred within 72 hours after the end of the last exposure, and the epithelium regained normal morphology. At 1 and 4 hours after the end of the treatment the frequency of S-phase cells along the crypt was greatly reduced and at the following intervals labelled cells occupied the region where differentiation occurs in control animals. During recovery labelled cell distribution showed a gradual return to normal. No substantial differences between the effects of total doses of 6 and 12 Gy were shown except for a greater reduction in crypt epithelial cells at the early time intervals after the larger dose.

Key words: Radiation biology, digestive system studies; rats, small intestine, irradiation, multiple fractions, cellular alterations.

The need to improve the therapeutic ratio between healthy and neoplastic tissues has led us to investigate the morphologic and biochemical functional modifications induced by new types of fractionation. Total dose, dose per fraction and time interval between fractions are parameters which affect radiation responses.

In previous studies changes in the small intestine have been assessed after total doses of 6 and 12 Gy given in 3 Gy fractions every 12 h (7, 8, 11, 12). The small intestine tolerated this treatment quite well. After the acute damage the epithelium quickly recovered and returned to conditions very similar to those of controls.

The present investigation deals with the same total doses as previously used but given in three 2 Gy fractions with one fraction every 4 h and in some groups followed by a second similar course of irradiation started after an interval of 16 h. This dose sequence has been used in clinical practice in patients with tumours of the nasopharynx (18).

It was previously demonstrated that after a single 8 Gy dose the response could be modified by choosing different times of the day for the irradiation (6, 13). In the present investigation therefore, the radiation treatment always was started at the same hour of the day and also the sacrifice times were strictly controlled.

Material and Methods

One hundred and seven female 10 to 12 week old Wistar rats weighing 180 to 200 g were used. The animals were kept at a constant light/darkness cycle (6.30 a.m. to 6.30 p.m.), and provided food and water ad libitum. Experimental conditions were similar to those used in previous studies (2–4, 7, 8, 11–14).

The ether-anaesthetized animals were exposed against the abdomen (field 5 cm × 5 cm) with the beam from a telecobalt unit (dose rate 0.8 Gy/min). The dose was calculated at midline.

In both groups the irradiation started in the late afternoon, because rats are nocturnal animals. Six control groups of 8 animals each were killed at the same time of the day as the irradiated animals.

In the first experiment a total dose of 3×2 Gy was administered with one fraction every 4 h and groups of 3 animals were killed at 1, 4, 12, 36, 72 h, and 5 and 11 days after the last fraction. In the second experiment a dose of 6×2 Gy was administered split in 2 similar courses with an interval of 16 h between the courses. Groups of 5 animals

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were killed at 1, 4, 24, 36, 72 h, and 5, 7, 11, 19 and 29 days after the last fraction. Animals from the 11th day after irradiation were killed at 2 a.m., 8 a.m., 2 p.m. and 8 p.m. in order to evaluate the return to periodical oscillations of parameters normally showing a circadian dependence.

One hour before being killed all animals were injected intraperitoneally with 3.7 MBq ^{3}H-thymidine (specific activity 74 GBq/mmol). The treatment of the small intestine for the histologic obeservations of the proximal jejunum, the counting of epithelial cells and the computer program have been reported previously (2). Qualitative morphologic observations were carried out and the following quantitative parameters were evaluated: a) number of epithelial cells in the crypts, b) labelling index, c) mitotic index, d) frequency distribution of labelled cells along the crypts.

The left side of 40 to 50 well aligned crypt-villus formations were counted for each animal. Values were expressed as percentages of controls killed at the same hours; ±SEM values were calculated. Student's t-test was used to compare the differences between irradiated and control animals. In three groups, when the counts were low due to morphologic alterations, only mean values were calculated.

Results

Morphologic observations. Post-irradiation morphologic changes in the small bowel behaved in the well-known way (20, 21) and were dependent on the time interval between the beginning of treatment and sacrifice.

After the 3×2 Gy fraction regimen nuclear and cytoplasmic alterations involved only the epithelial cells of the crypt until 12 h after irradiation, whereas at 36 h the whole crypt appeared damaged. Five days after irradiation the epithelial morphology was again similar to that of controls although a few altered cells were still present in the villi.

After the 2×3×2 Gy fractionation scheme alterations involved the whole crypt and more than half of the villus already at the first time intervals after irradiation. At 24 and 36 h the epithelium had reduced height with large areas where cell alignment had disappeared. Villi appeared stumpy and conglutinated with some vacuolated cells. At 72 h the cells of the crypts were normal and very numerous, although cells with globous nuclei were present in the upper part of the villi. At later time intervals the epithelium presented regular morphology.

Epithelial cells of the crypt. As early as 1 and 4 h following the 3×2 Gy fractionation regimen the number of epithelial cells of the left side of the crypt was depressed to about 60 per cent of control values (Fig. 1).

From 72 h on, this parameter returned to control levels and remained constant throughout the study.

After 2×3×2 Gy the reduction of crypt cell number at 1 and 4 h was significantly higher ($p<0.01$) than after 3×2 Gy. At 24 and 36 h the number of crypt cells had increased

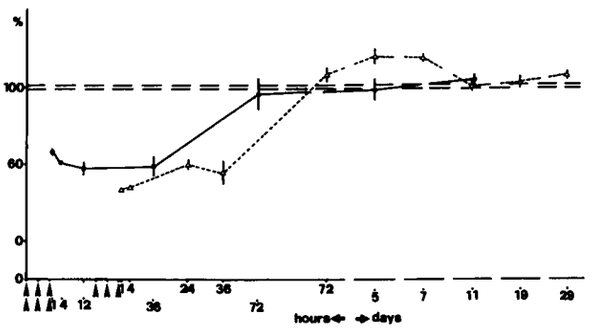

Fig. 1. Mean number ±SE of crypt cells in the proximal jejunum after 3×2 Gy (—) and 2×3×2 Gy (– – –). The values are expressed as percentages of controls killed at the same time of the day.

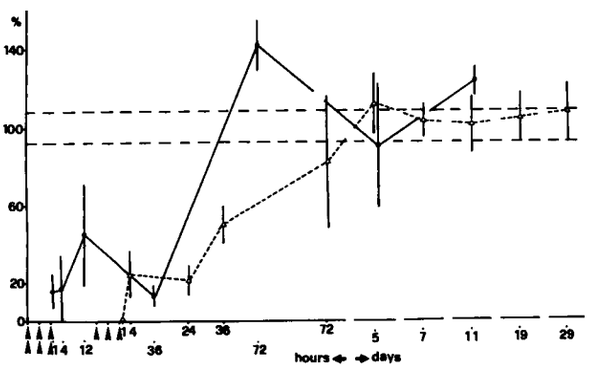

Fig. 2. Mitotic index in the proximal jejunum after 3×2 Gy and 2×3×2 Gy (see Fig. 1).

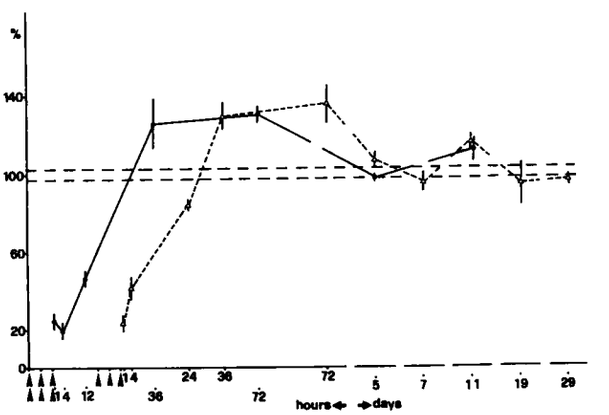

Fig. 3. Labelling index in the proximal jejunum after 3×2 Gy and 2×3×2 Gy (see Fig. 1).

compared with previous intervals, and at 72 h it was significantly higher than in the controls ($p<0.05$). At 5 and 7 days after the last fraction, peak values were reached ($p<0.01$) and subsequently the number of epithelial cells returned to normal values.

Mitotic index. The mitotic index after 3×2 Gy was very

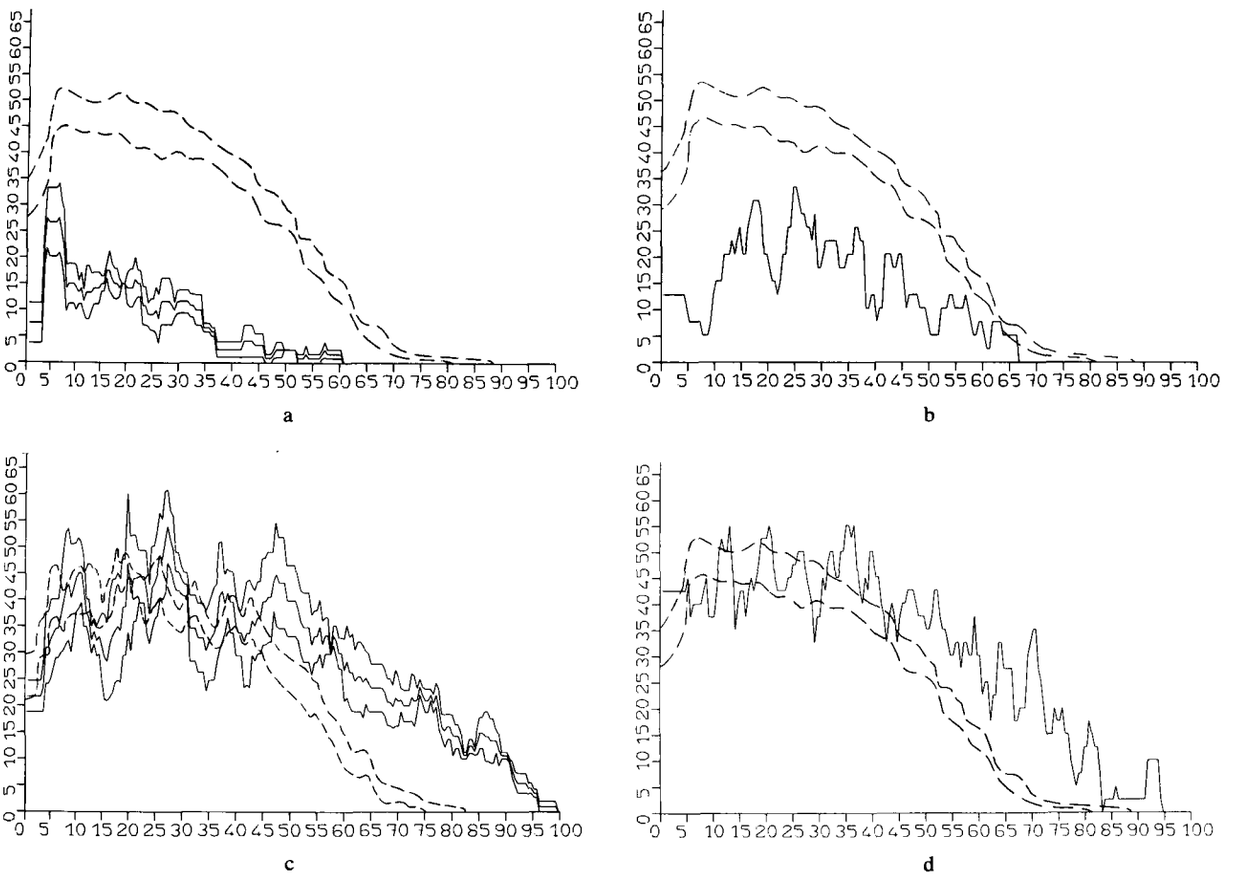

Fig. 4. Labelled cell distribution in the left side of the crypts of animals killed 1, 12, 36 and 72 h after 3×2 Gy (a, b, c, d, respectively). The mean value ±SE is reported in irradiated and in control animals (dotted band). The number of epithelial cells was 25±0.4, 22±1.0, 23±1.9 and 37±3.0, respectively. The positions of cells along the crypt are reported in the abscissa and the frequency of labelled cells in the ordinate.

low from 1 to 36 h after the last fraction (Fig. 2). At 72 h a statistically significant increase was observed ($p<0.01$) compared with control levels. At 5 days the values were similar to those of non-irradiated animals and at 11 days increased values ($p<0.05$) were observed.

After the 12 Gy total dose the mitotic index was about zero 1 h after the end of the treatment while at 4 and 24 h values of about 20 per cent of controls were observed. The return to control values was slower than after 6 Gy and occurred first from day 5 after the last fraction. The values then remained constant.

Labelling index. After 3×2 Gy there was a drop in the labelling index to about 20 per cent of control values at 1 and 4 h after the last fraction (Fig. 3). Subsequently at 36 and 72 h there was a rapid increase up to levels significantly higher ($p<0.02$) than in non-irradiated animals. At 5 days the values were similar to those of controls but exceeded them again ($p<0.05$) at 11 days after irradiation.

After 2×3×2 Gy the index showed an initial reduction and a curve similar to that after 3×2 Gy. Significantly higher values than in controls were thus observed at 36 and 72 h ($p<0.02$). The values then returned to control limits, but were again increased on day 11. The labelling index of animals killed at 4 different times at 11 days showed levels significantly higher than controls with a similar behaviour at different times of the day.

S-phase cell distribution. The localization and frequency of labelled cells along the crypt in control animals killed at different times of the day were exactly similar to those observed in previous studies (2). At the base of the crypt, where there are stem cells and differentiated cells, the frequency of S-phase cells was low. Higher up, the frequency increased and reached a level of 50 to 60 per cent in the lower half of the crypt. In the upper third of the crypt, where differentiation occurs with synthesis of the brush border enzymes, S-phase cells were practically absent.

The main difference between control animals killed at different times of the day was a higher frequency of S-phase cells in the lower part of the crypt in animals killed during the dark period; this difference was particularly evident between the 8 a.m. and the 8 p.m. groups (2).

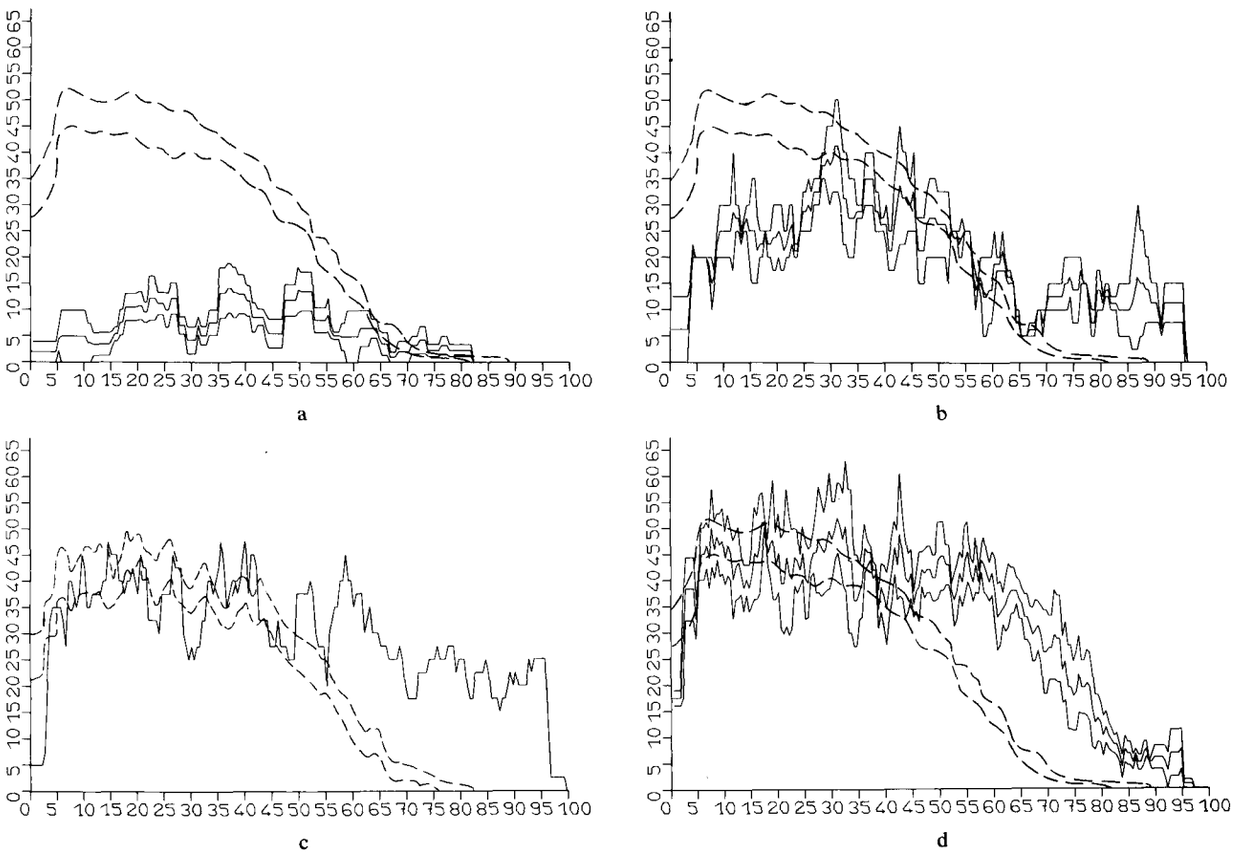

Fig. 5. Labelled cell distribution 1, 24, 36 and 72 h after 2×3×2 Gy (a, b, c, d, respectively). The number of epithelial cells was 18±0.3, 23±0.8, 22±2.4 and 41±1.0, respecively (see Fig. 4).

a) 3×2 Gy fractionation. One hour after the last fraction the frequency of labelled cells was mildly reduced in the lower 10 per cent of the crypt and markedly reduced in the rest of the crypt (Fig. 4 a). In the upper 40 per cent of the crypt labelled cells were absent. An even higher reduction with the same characteristics was observed at 4 h. At 12 h the labelled cell frequency had decreased at crypt bottom and increased in the median and upper part, although the values were still lower than in controls (Fig. 4 b). The frequency had markedly increased at 36 h when – although the number of crypt cells was the same as at 12 h – the distribution in the lower half was similar to that in controls, whereas it was significantly increased in the upper half (Fig. 4 c). Labelled cells were present also in the upper third, where under normal conditions differentiation occurs. At 72 h the frequency was still increased in the upper part of the crypt (Fig. 4 d). At 5 and 11 days the curves were similar to those of corresponding controls. At 11 days the labelled cell frequency was again higher in the upper half of the crypt.

b) 2×3×2 Gy fractionation. One hour after the last fraction S-phase cell frequency was evenly reduced to values between 5 and 15 per cent of controls in about 80 per cent of the whole crypt (Fig. 5 a). At 4 h there was an increase in distribution frequency in the third intermediate of the crypt. This increase was particularly marked at 24 h with high frequency of labelled cells also at the villus junction (Fig. 5 b). In the lower third of the crypt an evident reduction persisted compared with control values. At 36 h distribution the frequency was similar to that of corresponding controls in the lower half of the crypt whereas in the upper 40 per cent of the crypt it remained reduced to about 20 per cent (Fig. 5c).

After 72 h the pattern was similar, with reduction of labelled cells in the area of the villus junction (Fig. 5 d). At 5 days the curve was even more similar to that of non-irradiated animals even if the frequency was somewhat reduced in the lower part of the crypt and somewhat increased in the upper third. At 7 days the distribution curve appeared quite similar to that of controls.

In animals killed every 6 h, at the 11th day, the patterns were reasonably similar to those of controls killed at corresponding times of the day (Fig. 6). In groups killed at 2. a.m. and 8 p.m. only a slight relative increase in frequency in the upper third of the crypt was observed, whereas in animals killed at 8 a.m. and 2 p.m. the frequen-

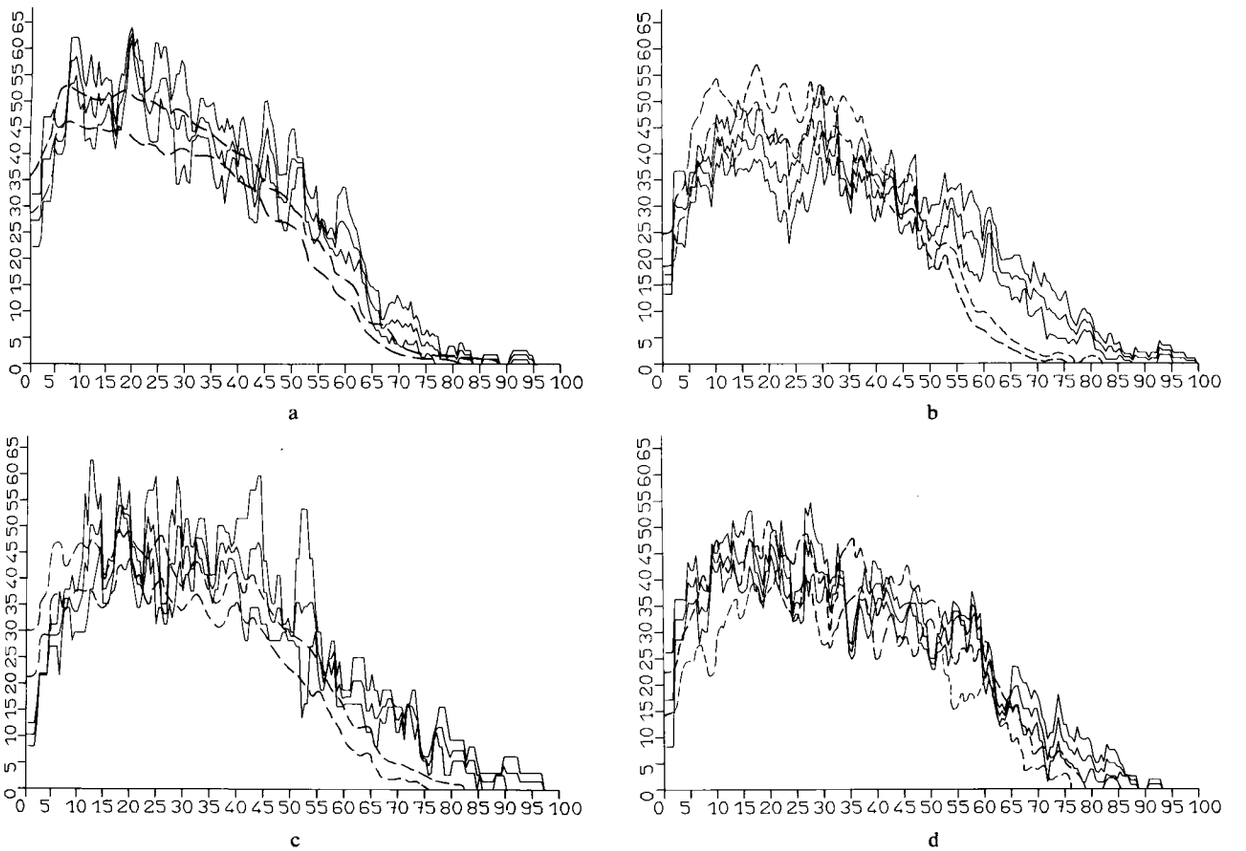

Fig. 6. Labelled cell distribution in animals killed 11 days after 2×3×2 Gy at 2 and 8 a.m. and 2 and 8 p.m. (a, b, c, d, respectively). The number of epithelial cells was 38±0.9, 42±0.4, 41±1.8 and 45±0.4, respectively (see Fig. 4).

cy was somewhat reduced in the lower third of the crypt and increased in the upper third. Similar behaviour was observed also 19 and 29 days after the last fraction.

Discussion

In previous reports the early cellular changes in the small intestine of the rat after a single dose of 8 Gy and after total doses of 6 and 12 Gy fractionated with 3 Gy every 12 h were described (3, 4, 7, 8, 11, 12, 14). The data from previous reports obtained from studies on brush border enzyme and goblet cells suggest an early differentiation, a process by which proliferative cells, unable to carry on mitosis due to radiation damage on non-vital genes, still are able to differentiate (22, 24, 25). It was demonstrated that the extension of the proliferative compartment (indicated by S-phase cells labelled by ^{3}H-thymidine) corresponded to the peak in epithelial damage, when the crypt-villus formation had lost its regular structure, and in many crypts repopulation was evident. At the same time the epithelium was characterized by almost total absence of brush border enzyme activity (5, 10, 14, 15) and of goblet cells (9). Subsequently during recovery, when the S-phase cell compartment decreased, the brush border enzymes showed a partial increase (11, 14).

The aim of the present study was to investigate early cellular changes and repair in rat small intestine after a new irradiation protocol and compare the effects with those after previously studied protocols. The new protocol implied 1 or 2 courses of irradiation with accelerated fractionation (3×2 Gy with a 4 h interval) and with a 16 h interval between the courses, when 2 courses were given. The total doses were 6 and 12 Gy, respectively. Of special interest were the tolerance and repair capacity of the epitelium and the effectiveness of the 16 h interval for the recovery.

Both after 3×2 Gy and 2×3×2 Gy there was a dramatic decrease in the number of crypt cells and in the mitotic index during the first 36 h after exposure, somewhat more marked after the larger total dose. After both regimens there was a return to normal values at 72 h. Already at 36 h an increase of the labelling index was observed, indicating proliferative activity and repair.

The difference between the effects of 3×2 Gy and 2×3×2 Gy was quite small. The larger total dose gave,

however, a greater reduction in crypt cells at early time intervals. There was also a longer duration of the extension of the proliferative compartment (labelled cells) into the differentiation area after the larger dose, indicating incomplete repair during the time interval between the 2 courses of irradiation.

The effects observed in the present study were, concerning the general pattern of response, strikingly similar to those previously reported after a single dose of 8 Gy (3, 4, 14). As could be expected from other fractionation studies, however, both 3×2 Gy and 2×3×2 Gy regimens were followed by somewhat less marked cellular changes and more efficient repair than a single dose of 8 Gy.

Of greater interest was a comparison with the effects observed after 2×3 Gy and 4×3 Gy given with one fraction every 12 h (7, 8, 11, 12). The number of crypt cells in the fractionation with 2 Gy doses had a behaviour similar to the others, while the mitotic and labelling indices showed a more evident decrease at the first intervals. Furthermore the frequency of labelled cells in the crypt was lower in the present protocol.

The fractionation regimen used in the present investigation was well tolerated by the small intestine and allowed rapid and obviously complete repair of the early cellular changes, which may be of interest from a clinical point of view. It must be underlined, however, that the total radiation doses delivered in this study were considerably lower than doses ordinarily used in clinical radiation therapy. The investigation has, however, further elucidated the dynamics of early radiation injury and recovery in the small intestine.

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