Modifications of S-phase cell distribution in the intestinal crypts after multiple daily fractionation.

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MODIFICATIONS OF S-PHASE CELL DISTRIBUTION IN THE INTESTINAL CRYPTS AFTER MULTIPLE DAILY FRACTIONATION

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The introduction of unconventional schedules of dose administration has justified an investigation on the behaviour of tissues whose alterations may affect the radiation dose used in tumor therapy.

An analysis of the structure of the proliferative compartment in tissues with high proliferative activity and turnover may supply useful information on the extent of the injury induced by physical and chemical agents as well as on recovery.

Previously, CAIRNIE (1967), AL-DEWACHI et coll. (1979) and BECCIOLINI et coll. (1983 a) demonstrated that epithelial cells capable of incorporating 3H Thymidine in the proliferative compartment of the small intestine presented preferential localization. It was also demonstrated that the distribution of labelled cells in the crypts showed different values related to the different times of the day when control animals were killed.

The differences in S-phase cell distribution produced by ionizing radiation after a single session have already been reported (BECCIOLINI et coll. 1983 b, c). This parameter indicates the injury in the proliferative compartment and the efficiency of cell recovery, and indirectly of functional recovery. In the present experiment the modifications of labelled cell distribution were analyzed in animals exposed to multiple daily fractionation.

This experiment was part of a wider research programme aimed at investigating new dose administration schedules in an attempt to improve the results of radiation therapy. Biochemical, qualitative and quantitative morphologic parameters were taken into account.

Material and Methods

A series of 105 female Wistar rats, 10 to 12 weeks old and weighing 180 to 200 g were used. They were kept at a constant L/D cycle (6.30 a.m. to 6.30 p.m.), with water and food ad libitum. Every other day in the initial hours of the light period the same person cleaned the cages and supplied the food. Two groups of ether anesthetized animals were exposed on the abdomen (field 5 cm × 5 cm) to a telecobalt unit (dose rate 0.8 Gy min^{-1}). The dose was calculated at midline. The first group was exposed to a total dose of 6 Gy split into 2 fractions of 3 Gy each with a 12 h interval, and groups of 3 animals each were killed at 1, 4, 12, 36, 72 h, and 5 and 11 days after the last fraction.

In the second group a total dose of 12 Gy was administered in 4 fractions every 12 h. Groups of 3 to 4 animals each were killed 1, 4, 24, 36, 72 h, and 5, 7, 11, 19 and 29 days after the end of exposure. The animals of the 11 day interval were killed at 2 a.m., 8 a.m., 2 p.m. and 8 p.m. in order to evaluate the return to circadian oscillations.

Five control groups of 6 to 8 animals each were killed during the day at intervals corresponding to the specific times of the irradiated groups. In both groups the irradiation started at noon. The total time of irradiation and that of killing was strictly limited to 50 min for each group.

One hour before killing all animals were injected intraperitoneally with 3.7 MBq (100 μCi) of 3H Thymidine (specific activity 74 GBq/mmol).

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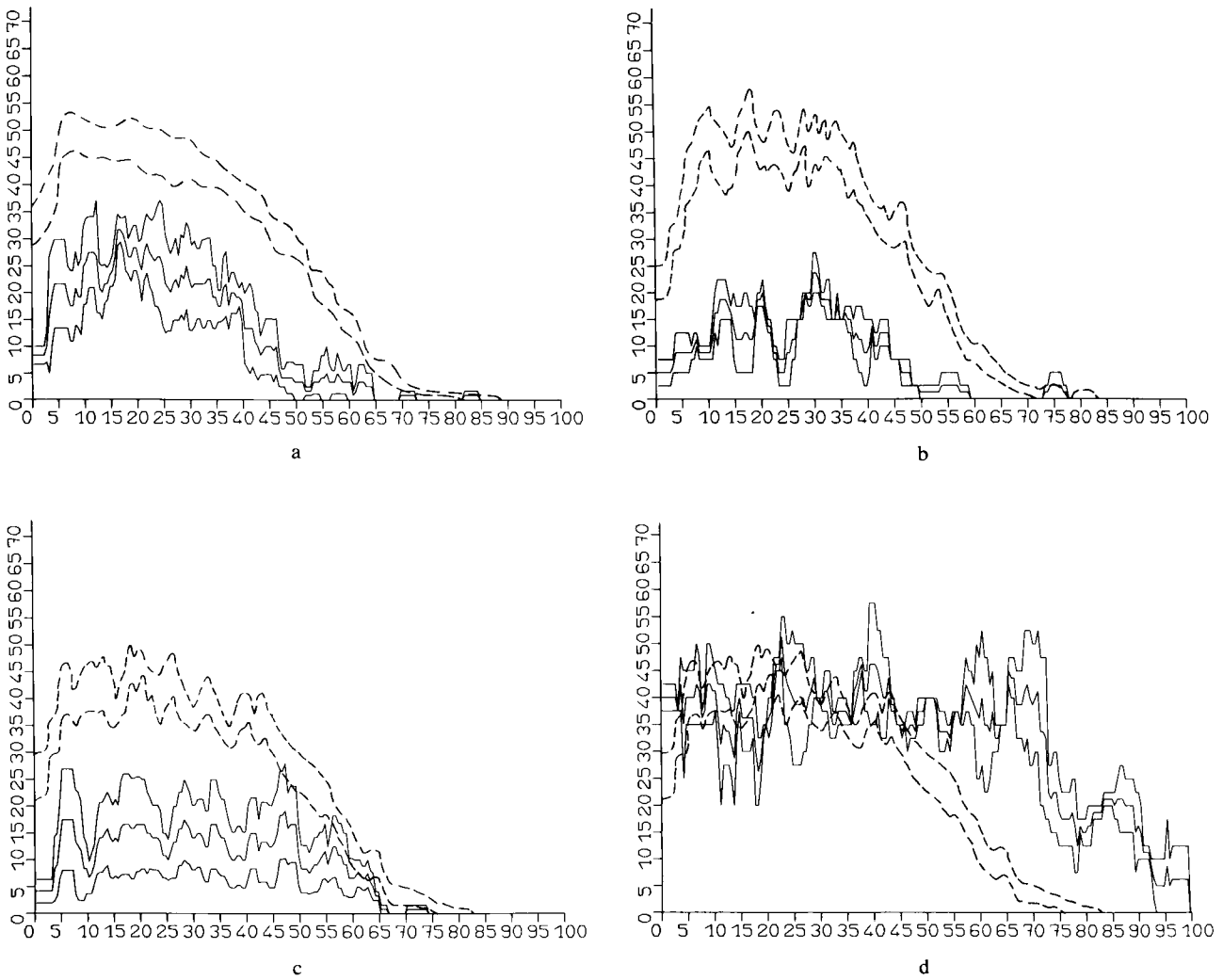


Fig. 1. The frequency as per cent of labelled cells between the highest and lowest limits of SEM in the theoretic crypt of animals killed at a) 1 h, b) 4 h, c) 12 h and d) 36 h after the last fraction of 3 Gy×2. Dashed line indicates the curve of controls killed at the same time of the day. The mean number of epithelial cells in the left side of the crypt was 29, 27, 23 and 25, respectively.

The treatment of the small intestine, the counting methods and the computing programme in Fortran language have been reported previously (BECCIOLINI et coll. 1983 a).

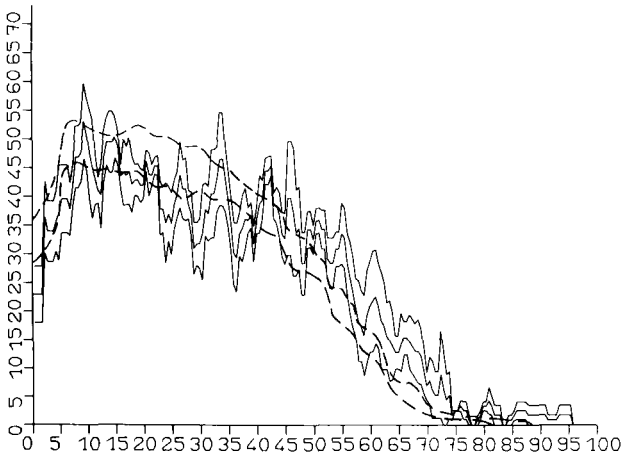
In order to evaluate the behaviour of the functional compartment and the differentiation process, invertase activity was assayed in the 5 segments into which the small intestine was cut. After homogenization with distilled water at 10 per cent (weight/volume), enzyme assay was carried out in duplicate on the supernatant after centrifugation at 900 × g, according to DAHLQUIST's (1964) technique.

Mean values ± SEM were calculated and Student's t-test was used to compare the differences between irradiated and control animals.

Results

Morphologic observations. At the initial intervals morphologic alterations after 3 Gy × 2 fractionation were limited to the crypts; then, because of turnover, the heaviest modifications appeared and progressively involved larger portions of the villi. From 72 h after exposure onwards, the epithelium again showed a morphology similar to that of the controls, although some altered cells were still present along the formation.

As treatment with 3 Gy×2×2 fractionation was longer, alterations involved both proliferative and differentiated compartments already from the early killing intervals, and they appeared much more marked when compared with the other fractiona-



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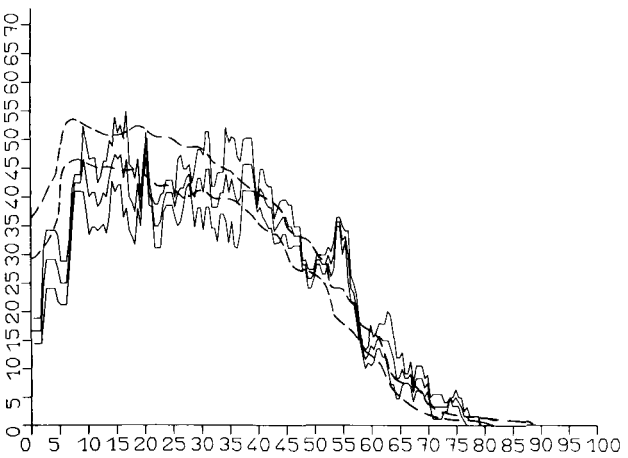

b

Fig. 2. The frequency as per cent of labelled cells in the theoretic cypt of animals killed at a) 72 h and b) 5 days after the last fraction of 3 Gy×2. The mean number of epithelial cells in the left side of the crypt was 42 and 41, respectively (cf. Fig. 1).

tion. Alterations also involved the upper halves of the villi; crypts were thin with globous, widely spaced nuclei; some formations were separated by areas having stroma only. No mitotic activity was observed.

At the following intervals the injury of the epithelium progressed and at 36 h the villi appeared short and conglutinated, with globous cells all the way to the top. At 72 h, very high proliferative activity was evident in the crypts. However, at this interval and at the next, recovery did not seem to proceed homogeneously. In fact, in the same specimen and in the animals belonging to the same group, severe cell alterations were observed adjacent to regenerating crypts. At the following intervals, a morphology similar to that of the controls was observed.

S-phase cell distribution. Distribution of cells which took up 3H Thymidine presented a characteristic pattern, as is described elsewhere (BECCIOLINI et coll. 1983 a), in control animals. The frequency of labelled cells at the bottom of the crypt was very low in the initial positions; after that, it increased rapidly, reaching the maximum value within 50 per cent of the crypt. The frequency decreased rapidly to 5 per cent in the intermediate third of the crypt, and later the percentage of S-phase cells was nil above 80 per cent of the total height.

Statistically significant differences in labelled cell distribution were observed in control animals killed at different times of the day (BECCIOLINI et coll. 1983 a). When compared with the groups killed at night, the animals killed in the late afternoon showed a lower frequency of labelled cells in the lower part of the crypts, whereas the frequency increased in the differentiating compartment area.

The distribution curves of controls killed at different times of the day, in this experiment, showed the same values as previously observed.

3 Gy × 2 fractionation. One hour after the last fraction (Fig. 1 a) the frequency of S-phase cells was markedly reduced in the entire lower half of the crypts, but the shape of the curve appeared similar to that of the corresponding controls. At 4 h (Fig. 1 b), a further reduction of labelled cells occurred in the lower third of the crypts and the presence of cells which had taken up 3H Thymidine was unlikely in the higher 40 per cent.

The distribution appeared homogeneous, in the animals killed at 12 h, as high as 60 per cent of the height of the formation (Fig. 1 c). The frequency was about one third as high as those found in the corresponding positions in the control animals.

At 36 h after the last fraction (Fig. 1 d) S-phase cell frequency in the lower half of the crypts reached control levels and at the same time an increase in epithelial cells was noted. In the upper half, the frequency was significantly higher and labelled cells were present at the crypt-villus junction.

The curve for the labelled cell distribution in the crypts of animals killed at 72 h (Fig. 2 a) was similar to that of controls except for the upper part where some increase in S-phase cells was observed.

Five (Fig. 2 b) and 11 days after exposure the distribution was practically the same as in controls

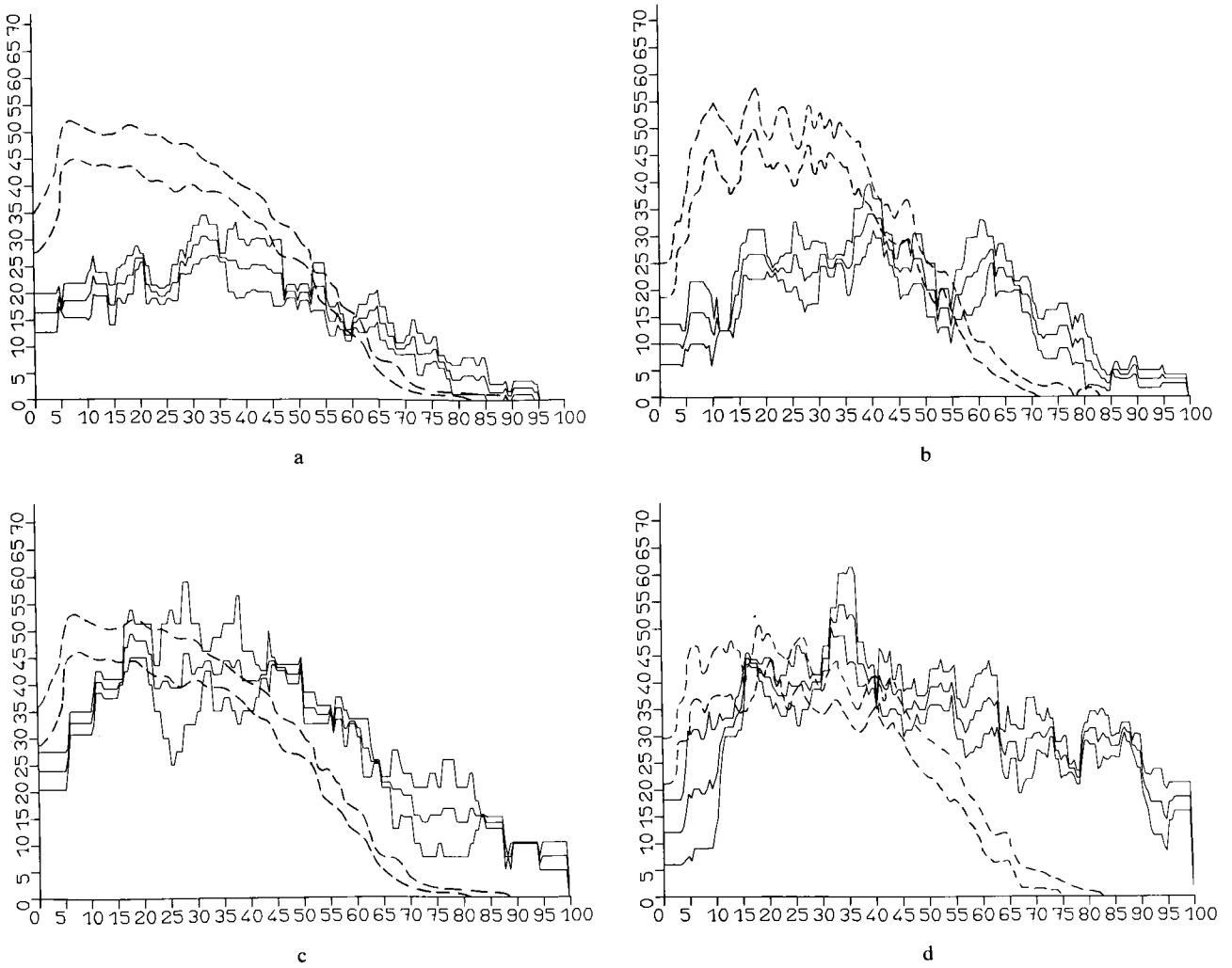

Fig. 3. The frequency as per cent of labelled cells in the theoretic crypt of animals killed at a) 1 h, b) 4 h, c) 24 h and d) 36 h after the last fraction of 3 Gy×2×2. The mean number of epithelial cells in the left side of the crypt was 19, 20, 18 and 21, respectively (cf. Fig. 1).

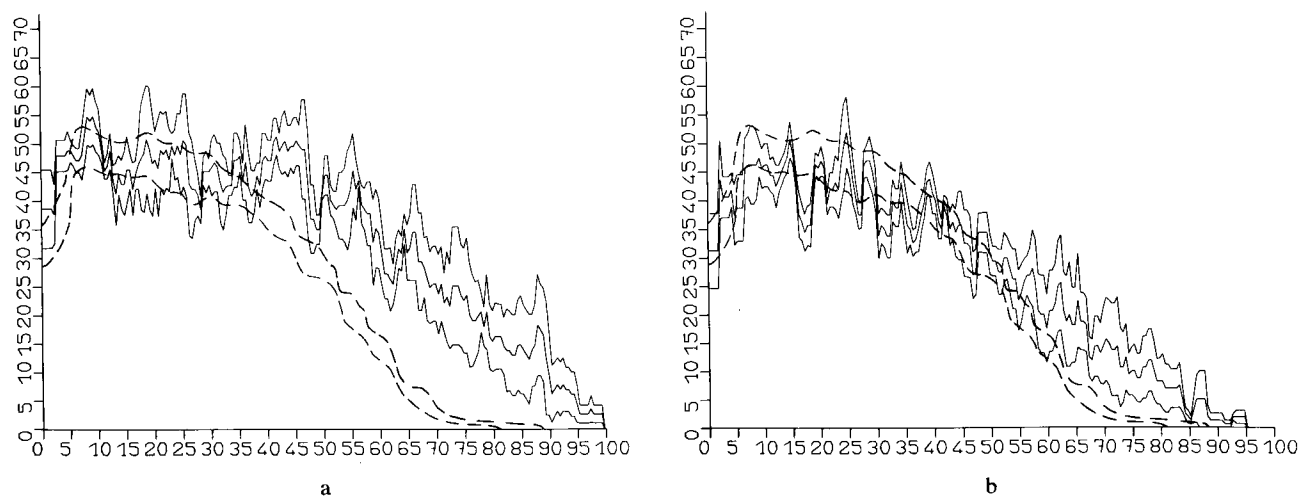

Fig. 4. The frequency as per cent of labelled cells in the theoretic crypt of animals killed at a) 72 h and b) 5 days after the last fraction of 3 Gy×2×2. The mean number of epithelial cells in the left side of the crypt was 41 and 43, respectively (cf. Fig. 1).

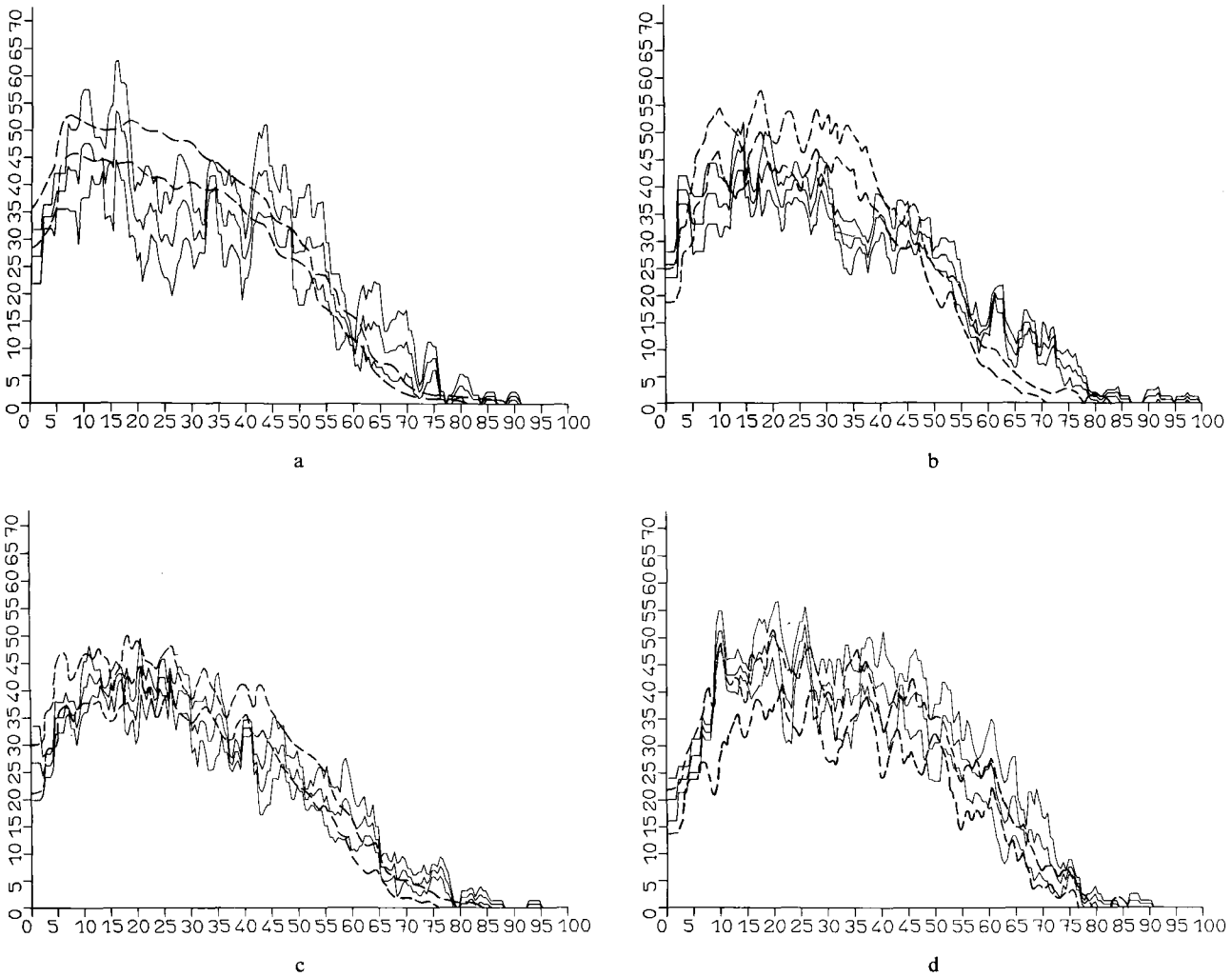

Fig. 5. The frequency as per cent of labelled cells in the theoretic crypt of the groups of animals killed at a) 2 a.m., b) 8 a.m., c) 2 p.m. and d) 8 p.m. with an 11-day interval. The mean number of epithelial cells in the left side of the crypt was 44, 38, 43 and 44, respectively (cf. Fig. 1).

apart from a slight decrease in frequency at the bottom of the crypt.

3 Gy×2×2 fractionation. In animals irradiated with this schedule of multiple daily fractionation the number of epithelial cells in the crypts was practically constant with values about half as high as in controls from 1 h to 36 h after the last fraction.

In groups killed 1 and 4 h after exposure (Fig. 3a, b), labelled cell frequency in the lower third of the crypt was less than half as high as in controls. Values had increased significantly in the upper third, particularly in the 4 h group where labelled cells were also present at the villus junction.

At 24 h (Fig. 3c) a significant reduction in the labelled cell frequency was always observed in the first positions of the crypts. In the area immediately above that, distribution was similar to the value in the controls whereas the frequency showed a statistically significant increase in the upper part. When the animals were killed 36 h after the last fraction (Fig. 3d) the bottom of the crypts still showed reduced frequency of labelled cells whereas further extension of these cells was observed in the differentiating compartment. At 72 h (Fig. 4a) epithelial cells were about twice as many as at previous intervals and S-phase cell distribution was similar to the controls in the lower 40 per cent of the crypts. Although reduced, when compared with the previous interval, the frequency was still significantly higher than in controls in the upper part.

A further decrease of this phenomenon was observed at 5 (Fig. 4b) and 7 days.

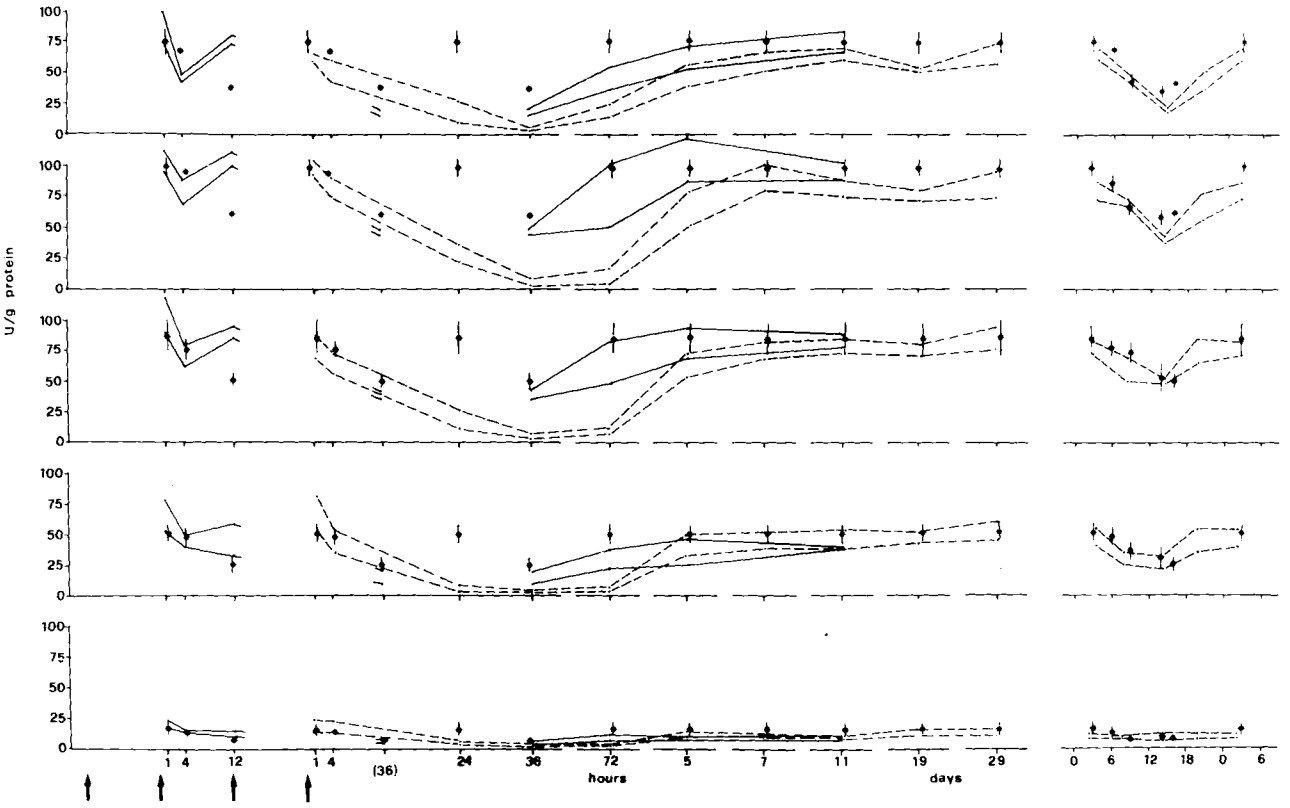

Fig. 6. Invertase activity as U/g of protein in the five segments of the small intestine after 3 Gy×2 and 3 Gy×2×2.

In the groups of animals killed every 6 h at the 11 day interval, to evaluate any possible return to circadian oscillations, the general pattern was very similar to that of the controls killed at the same time of the day (Fig. 5). The differences consisted of a reduction in labelled cell frequency between 20 and 40 per cent of the crypt height in those groups killed at 2 and 8 a.m. and of the extension of the proliferative compartment into the upper half of the crypt. These curves appeared similar to those of controls killed in the afternoon. At 2 and 8 p.m. the curves were similar to the corresponding controls except for a slight increase in labelled cell frequency in the areas closest to the villi. The same behaviour was oberved in animals killed 19 and 29 days after exposure.

Invertase activity. The activity of this brush border enzyme was determined in order to investigate the condition of the differentiating compartment where this molecule is synthetized for the first time. The behaviour of the invertase activity after irradiation was fairly homogeneous in the five segments into which the small inestine was cut (Fig. 6). At the first two intervals following 3 Gy×2 fractionation the values were similar to those in the corresponding controls whereas at 12 h the activity was significantly higher ($p<0.01$). At 36 h after irradiation the levels were lower than in the controls but only in the first two segments were the differences statistically significant ($p<0.02$). From this interval onwards, the values increased but not homogeneously among the animals of the same group and at 5 days they were at control levels.

At the first two intervals after 3 Gy×2×2 fractionation, the invertase activity persisted at control levels and gradually decreased till it reached values close to zero at 36 and 72 h. With this dose, in the initial two segments, the activity was significantly lower as late as 5 days after exposure. The same result was observed 11, 19 and 29 days after irradiation. In the other segments of the same small intestine the values were at minimum control values.

The animals killed at close intervals 11 days after irradiation showed oscillations again during the 24 h cycle with highest levels during the dark period and lowest in the afternoon, the same as in controls. The

activity appeared similar to the lowest levels of the corresponding controls or significantly lower.

Discussion

A system of multiple daily fractionation has recently been introduced in clinical trials for the treatment of tumors, although dose per fraction, their number, and the splits among fractions were established somewhat empirically.

The aim of this investigation was to analyze the behaviour of healthy, highly proliferative tissues after exposure to 2 daily 3 Gy fractions, which, if administered in a single session, induced acute intestinal death in about 35 per cent of the animals (BECCIOLINI et coll. 1972, 1974).

The distribution of labelled cells in the crypt was used to evaluate the condition of the proliferative compartment. In control animals the percentage of labelled cells in the crypts was high because the S-phase lasts for about 60 to 70 per cent of the cell cycle time. A different distribution of S-phase cells in the crypt had already been demonstrated and it seemed to correspond to precise morphologic and functional characteristics (CAIRNIE, AL-DEWACHI et coll.). In the upper third of the crypt no labelled cells were observed; in fact, in this part, cell differentiation occurred.

Previous studies carried out after 8 Gy in a single abdominal irradiation administered at different times of the day had demonstrated that at the first intervals a block in 3H Thymidine uptake occurred (BECCIOLINI et coll. 1982, 1983 b, c), which was particularly evident in the lower half of the crypts. From 20 h after exposure the synthetic activity of DNA in the lower part of the crypt was recovered, but labelled cells were observed even in the area when cell differentiation normally occurs.

This result was particularly evident at 72 h when labelled cells were localized in the whole crypt. At subsequent intervals the S-phase cell frequency gradually tended to return to control values. Although recovery appeared earlier in all groups at 11 days and later, in the animals irradiated at the end of the dark period, labelled cell distribution was similar enough to controls.

With multiple fractionation, the effect of radiation was different, as regards the time of its appearance, owing to repair and to recovery phenomena occurring during the intervals between exposures, but the general behaviour appeared to be similar to that induced by the single dose.

The 6 Gy total dose demonstrated, from the first fractionation, a block of 3H Thymidine uptake and therefore of cellular proliferation. The fractionated exposure produced only a progressively higher injury but no modification of behaviour.

The injury appeared less severe and the recovery quicker with the lower dose.

The severity of alterations after the 3 Gy×2×2 fractionation appeared lower than that induced by a single dose of 8 Gy. The effectiveness of the recovery phenomena at the end of irradiation was well evident comparing the two fractionation curves at the early killing intervals.

The time needed for a return to a distribution similar to that of the controls was 5 days for the total dose of 6 Gy and 11 days for the higher dose.

The new steady state was characterized by a statistically significant increase of epithelial cells in the crypt, particularly with the 12 Gy total dose.

In the animals at the 11 day interval, killed every 6 h, the curves were at all intervals similar to that of the afternoon controls; in fact all the curves still had a certain extension of S-cells in the differentiating area. The same feature was observed at 19 days.

The return to normal morphology did not correspond to a normal functional capacity, assessed through invertase activity. This discrepancy between morphologic and enzyme data was particularly evident at 72 h when the epithelium was returning to normal and values for the enzyme activity were close to zero.

At 11 days, when the distribution curves of labelled cells were near the normal behaviour, invertase activity presented higher values during the dark period than in the light one, almost the same as circadian oscillations of controls.

With this fractionation, the return to enzyme activity similar to that of the controls appeared earlier than with the 8 Gy single dose. The comparison between multiple daily fractionation and 8 Gy in a single session administered at noon demonstrated a similar progression of the injury. In fact, adding the length of the treatment (36 h) to the multiple fractionation, the distribution curves of S-phase cells were similar to those obtained with the single dose.

SUMMARY

The effects obtained by multiple daily fractionation (3 Gy×2 or 3 Gy×2×2) on the distribution of S-phase cells along the crypt of the small intestine were investigated.

The frequency of labelled cell distribution was reduced at early intervals; then the proliferating compartment gradually extended to the villus junctions. During recovery labelled cell frequency in the lower half of the crypts returned to control levels, while labelled cells were present in the differentiating area. With lower total dose modifications were milder and, as early as 72 h before exposure, distribution was already similar to controls. Invertase activity showed an initial increase and a higher reduction during acute damage when fractionation with higher doses was used. A lack of return to normal activity was present even 11 days after exposure when, however, the characteristic circadian pattern was observed.

ACKNOWLEDGEMENT

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